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Effect of habitat fragmentation on levels and patterns of genetic diversity in natural populations of the peat moss *Polytrichum commune*

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Peat bogs represent unique ecosystems that are under particular threat from fragmentation due to peat harvesting with only 38% of the original peatland in Europe remaining intact and unaffected by peat cutting, drainage and silviculture. In this study we have utilised microsatellite markers to determine levels and patterns of genetic diversity in both cut and uncut natural populations of the peat moss *Polytrichum commune.* Overall diversity levels suggest that there is more genetic variation present than had previously been assumed for bryophytes. Despite this, diversity values from completely cut bogs were found to be lower than those from uncut peatlands (average 0.729 vs. 0.880). In addition, the genetic diversity was more highly structured in the cut populations, further suggesting that genetic drift is already affecting genetic diversity in peat bogs subjected to fragmentation.

Keywords: *Polytrichum commune*, bryophytes, fragmentation, genetic drift, microsatellites, peat bogs

Running title: Habitat fragmentation and genetic diversity in *Polytrichum commune*

1. INTRODUCTION

One of the key issues in ecological genetics today is the effect of habitat fragmentation on the biodiversity of a range of ecosystems (Saunders *et al*. 1991). Until recently, indicators of biodiversity have been limited to ecological parameters such as population dynamics and species richness. Recent advances in molecular genetic technology, however, have opened a new chapter in conservation efforts and results from molecular studies are becoming increasingly important in the conservation and management of a wide range of rare or threatened species (Haig 1998). Such techniques are of particular relevance to the analysis of plant populations, since plants vary widely in such factors as mode of reproduction (sexual vs. asexual; selfing vs. outcrossing), relative importance of seed and pollen movement and the role of dormancy in the re-establishment of populations (Young *et al*. 1996). Fragmentation of natural plant communities can have deleterious effects on the genetic diversity within a species since there will be a decrease in levels of gene flow, particularly over longer distances. The subsequent effects of genetic drift in small, isolated populations will lead to loss of diversity, leaving plants less able to adapt to changes in their environment and ultimately increasing the risk of extinction (Keller and Waller 2002). Fragmentation also affects the genetic structure of populations, with isolated fragments tending to be more genetically distinct than would be expected in a continuous population on a similar spatial scale.

Peat bogs represent unique ecosystems that are under particular threat from fragmentation. They are made up of around 92% water (Cabot 1999), most of which comes from rainfall, and are thus lacking in many of the nutrients required for plant growth. As a result, few plant species are found in bogs but those that are tend to be highly specialised, with several being endemic to the bog habitat. Despite the ecological value of bogs, however, many have been

severely impacted by peat cutting and one major consequence of this habitat destruction has been the fragmentation of natural populations of many plant species. European peat bogs, which are included in Annex 1 of the EU Habitats Directive, have suffered more than those in any other continent, with only around 188,000 km^2 (38%) of an estimated original area of 495,000 km^2 remaining (Raeymaekers 2000) and it is expected that harvested sites will rarely return to functional ecosystems after abandonment as drainage and peat extraction will have lowered the water table (van Seters and Price 2001). The potentially deleterious effects of peat cutting on such habitats are only now becoming apparent and it is obvious that suitable management strategies are crucial to the continued survival of peatlands.

One of the most important groups of plants found in bogs is the bryophytes (mosses, liverworts and hornworts). Bryophytes are unique among land plants in that the haploid gametophyte is the longer-lived, autonomous and more photosynthetically active generation. Diploid sporophytes are borne on gametophytic shoots and depend on them for water. This arrangement would appear to limit genetic diversity, as there can be no sheltering of recessive genes in the heterozygous state for the majority of their lifespan (Ennos 1990; Derda and Wyatt 1990). Prodigious numbers of spores are produced by most mosses and though some evidence suggests that dispersal rates decline exponentially with distance, there have been cases reported of long-distance dispersal events (Wyatt and Derda 1997). Consequently, diversity in mosses generally appears to be partitioned more among rather than within populations: on small geographic scales many moss populations display high levels of differentiation, suggesting that spore mediated gene flow is limited (Derda and Wyatt 1990, 1999; Wyatt and Derda 1997).

Polytrichum commune is the largest native moss in Ireland (Pilcher and Hall 2001). It is a dioecious species with a predominantly haploid life cycle, commonly found in the acidic soils of peat bogs. A long-lived perennial, it produces relatively small spores in prodigious

numbers (Hedderson and Longton 1996). As with all moss species, fertilisation depends on the presence of water and male gamete dispersal distances are therefore thought to be small, less than 20cm in most moss species, though recently in the related species *P. formosum* it was found that male gametes could disperse easily and frequently over distances larger than 1.5m (van der Velde *et al*. 2001a). *P. commune* can also colonise areas by asexual means through the spread of rhizomes or vegetative parts. Colonies commonly expand as the underground stems divide and grow, and the spread of these clones can effectively lower the level of diversity detected within the population. (Derda and Wyatt 1999)

In this study we have utilised microsatellite markers to determine levels and patterns of genetic diversity in both cut and uncut natural populations of *Polytrichum commune.* Microsatellite markers have previously been described for *P. formosum* (van der Velde *et al.* 2000, 2001b) and provide a more informative alternative to allozymes for population genetics studies in plants (for review see Powell *et al*. 1996). We have tested these markers for cross-species amplification in *P. commune* and used the information obtained to determine what effect, if any, fragmentation due to peat cutting has had on the genetic structure of populations.

2. MATERIALS AND METHODS

(a) *Sampling*

We sampled in total 256 discrete moss cushions of *P. commune* from four populations in Northern Ireland peatbogs (Figure1). Where possible, sampling was carried out at regularly spaced intervals. To ensure the sampling of different potentially clonal individuals, samples were taken from moss cushions separated by at least two metres. One leaf from each sample was sectioned, and the morphology of the apical cell of the lamellae determined according to Bijlsma *et al*. (2000) and Zouhair *et al*. (2000). Only samples of *P. commune* var. *commune* were used for subsequent analysis, and samples of *P. formosum* and *P. commune* var. *perigionale* inadvertently collected were discarded In total, 200 individual gametophytes were studied.

(b) *DNA extraction*

All samples were stored at –20^{o}C, and DNA was extracted from individual gametophytes using the Qiagen DNeasy® Plant Mini Kit, after an initial 6 min grinding at 30Hz using the Retsch MM300 mixer mill. DNA was quantified visually on 1% agarose gels stained with ethidium bromide and subsequently diluted to a concentration of 10ng/µl.

(c) *Polymerase chain reaction*

The primers used were those described by van der Velde *et al.* (2000 & 2001b) for use in *P. formosum*. These primers had been tested in other *Polytrichum* species (van der Velde and Bijlsma 2001) and primer pairs were selected which had produced a polymorphic band when tested in *P. commune*. In total nine primer pairs were tested, F-1, F-2, F-7, F-11, F-12, F-13, F-14, F-17, and F-21 (primer names from van der Velde *et al.* 2000 and van der Velde *et al.*

2001b). Of the primers tested only three (F-11, F-17 & F-21) gave clear, reproducible, polymorphic bands, and these were then used in all subsequent analysis.

All reactions were carried out on a MWG Primus thermal cycler using the following parameters: initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. PCR was carried out in a total volume of 10 µl containing 5 µl genomic DNA, 5 pmol ^{32}P-end labelled forward primer, 5 pmol reverse primer, 1X PCR reaction buffer (10 mM Tris-HCl [pH9.0], 50 mM KCl. 0.1% Triton® X-100), 2.5 mM $MgCl_2$, 1 U *Taq* polymerase (Promega). Products were resolved on a 6% denaturing polyacrylamide gel containing 1X TBE and 8 M urea after addition of 10 µl 95% formamide loading buffer. Gels were run at 70 W constant power for 2 hours, transferred to 3MM Whatman Blotting paper and exposed to x-ray film overnight at –20 °C.

(d) ***Data Analysis***

Allele sizes were scored using a 10 bp ladder and were checked by comparison with previously sized control samples. Diversity values based on allele frequencies were calculated using Nei's measure (1987). Interpopulation differentiation between the four populations studied was estimated using Φ, which gives an analogue of F_{ST} (Weir and Cockerham 1984) calculated within the analysis of molecular variance framework (AMOVA; Excoffier *et al*. 1992). The significance of fixation indices was tested using a nonparametric approach. All analyses were carried out using the Arlequin software package (Schneider *et al*. 2000).

3. RESULTS

(a) *Levels of within-population genetic diversity*

Of the nine primer pairs tested, six sets (F-7, F-12, F-13, F-11, F-17 and F-21) generated unambiguous scorable bands, three of which (F-11, F-17 and F-21) produced polymorphic bands. A total of 51 alleles was generated between the three microsatellite loci and data on the number and distribution of alleles at each locus for populations and sub-populations is given in Appendix 1.

Estimates of Nei's genetic diversity for individual loci are given in Table 2. Levels ranged from 0.742 (Larne-2 and Slievanorra-1) to 0.974 (Peatland's Park-1) for locus F-11 (average 0.847), 0.714 (Slievanorra-4) to 0.964 (Peatland's Park-4) for locus F-17 (average 0.856) and 0.455 (Larne-2) to 0.939 (Slievanorra-1) for locus F-21 (average 0.752). The overall average diversity level was 0.818. Multi-locus diversity values ranged from 0.782 (Larne-4) to 1.000 (several sub-populations: data not shown).

(b) *Levels of between-population genetic diversity*

No significant partitioning of total genetic diversity was observed at the population level (Φ_{CT} = -0.001; NS: Table 3). A small but significant amount (3.21%; $P < 0.001$) of the genetic variation was partitioned between subpopulations. Levels of genetic structure between subpopulations within individual populations are given in Table 4 and ranged from 0.00% (Sperrins: Φ_{ST} = -0.018; NS) to 13.53% (Larne: Φ_{ST} = 0.135; $P < 0.001$).

4. DISCUSSION

It is now accepted that fragmentation of natural populations can have deleterious effects on levels of genetic diversity in impacted populations (for reviews see Young *et al*.1996; Sork *et al*. 1999; Keller and Waller 2002). Bogs that have been subjected to peat harvesting represent one such ecosystem. In bryophytes, which comprise a high percentage of the typical bogland flora, these effects are expected to be exacerbated due to several factors, including the predominance of the haploid gametophyte phase in the bryophyte life cycle and the dependence on water to disperse male gametes. The aim of this study was to investigate what effect habitat fragmentation due to peat cutting has had on natural populations of the moss *Polytrichum commune* by comparing levels and patterns of diversity in a range of cut and uncut habitats. Until now, the application of high-resolution microsatellite markers to address the genetic consequences of population fragmentation has focused almost exclusively on tropical tree species (Dyanandan *et al.* 1999; White *et al*. 1999; Collevatti *et al*. 2001; White *et al.* 2002), which have long generation times and which tend to exhibit high levels of heterozygosity and outcrossing.

Overall levels of population diversity were found to be high, contrasting with the view that bryophytes should exhibit low levels of diversity due to their haploid phase-dominated life cycle when compared with seed plants (Ennos 1990; Stenoien and Sastad 2001). In a study carried out on Danish and Dutch populations of the related species *P. formosum* using microsatellites, 14 of 26 microsatellite loci were polymorphic (including the three loci used in this study) and gave diversity values (*H*) of between 0.322 and 0.432 for single loci (van der Velde *et al.* 2001b). Values of *H* for *P. commune* calculated from our data range from 0.729 to 0.844 (average 0.818), further confirming that natural populations of bryophytes may harbour more genetic diversity that had previously been thought. An earlier study on the

same Danish and Dutch samples using allozymes found an average diversity value of 0.034, which is approximately an order of magnitude less than that obtained using microsatellites (van der Velde and Bijlsma 2000) although these values included monomorphic loci. In their study of *P. commune* from North America and Europe, Derda and Wyatt (1999) found the diversity to be 0.061 and predicted that levels of diversity might be higher in *P. commune* than in other mosses because *P. commune* is unisexual and produces sporophytes regularly, occupies a variety of habitats and appears to have a wide ecological tolerance. Our study using microsatellites also found higher levels of diversity in *P. commune* than in a comparable study on *P. formosum* (van der Velde *et al.* 2001b). This is despite the potential effects of ascertainment bias, where markers are expected to display more variation in the species they were isolated from when compared with their utility in other species (Hutter *et al*. 1998).

Fragmentation is expected to result in a loss of genetic diversity in small isolated populations by the random process of genetic drift. A comparison of our diversity values obtained from cut, partially cut and uncut bogs would seem to confirm this: the lowest within-population average diversity values for all three loci were found in the Larne population (cut) and the highest were found in the Sperrins population (uncut). Values for the two partially cut bogs, Slievanorra and Peatland's Park, were intermediate. Studies on the moss species *Plagiomnium ciliare* using allozymes by Wyatt and co-workers (1992) found that populations from forests that had never been cleared or heavily logged exhibited significantly higher levels of genetic diversity than disturbed forests. They suggested that the reduction in genetic diversity could be due to a combination of founder effects and genetic drift, which were a consequence of recent habitat destruction that reduced population sizes and forced some colonies to re-establish from a limited number of surviving sources. Likewise, an allozyme study of *Swertia perennis*, a long-lived perennial fen specialist (a

habitat not unlike peatlands), examined 17 populations where the habitat had been severely fragmented and found that populations in small isolated fens had reduced genetic variability associated with increased levels of inbreeding (Lienert *et al*. 2002). Conversely, Thingsgaard (2001) did not observe a decrease in genetic diversity between fragmented and unfragmented populations of the moss *Sphagnum affine*, concluding instead that post-glacial recolonisation events were the major determining factors in shaping the genetic structure of the populations studied. It must also be borne in mind, though, that in the three cases described above, different levels of diversity across populations observed using non-neutral allozyme markers may be a result of differing selective pressures and may not be solely due to the effects of fragmentation, although the first two studies do largely reflect the findings of the microsatellites employed in this study which are generally considered to be effectively neutral (Jarne and Lagoda 1996).

A further expected consequence of fragmentation of plant populations is an increase in inter-population (or subpopulation) genetic divergence due to the random fixation of different alleles in different populations, particularly in taxa with limited gametic dispersal (Templeton *et al*. 1990). Over all the samples analysed in the present study, there was no significant partitioning of diversity between populations and only 3.21% of the total diversity was partitioned between subpopulations within populations. Comparably low levels of between-population diversity were also found in *P. formosum* (F_{ST} = 0.028; R_{ST} = 0.015) even though populations were separated by as much as 400 km (van der Velde *et al*. 2001b). This suggests that larger populations are able to maintain their high levels of genetic diversity and that genetic drift has not yet led to a gradual fixation of alleles and subsequent population differentiation. Although it is believed that members of the order Polytrichales may produce in excess of 5 million spores on average (Hedderson and Longton 1996) and that some of these may travel over relatively long distances, it is likely that a strongly leptokurtic dispersal

pattern would lead to the vast majority of spores being deposited close to the parent sporophyte. This is apparent when levels of genetic differentiation between subpopulations within individual populations are considered. No significant differentiation was found between the Sperrins subpopulations, which were taken from a continuous, uncut peat bog. The Larne population, however, which has been extensively cut and consists largely of a dispersed "mosaic" of uncut fragments, exhibited a high degree of differentiation between subpopulations ($\Phi_{ST} = 0.135$; $P < 0.001$). The Φ_{ST} values for the other two populations, which are comprised of a mixture of cut and uncut areas, showed a small but significant level of subpopulation differentiation.

The results of this study suggest that whilst natural populations of *P. commune* would appear to harbour higher levels of genetic variation than had originally been associated with bryophytes, the effects of habitat fragmentation – reduced within-subpopulation diversity and increased between-subpopulation differentiation – have already begun to become apparent where peat cutting has taken place. This is particularly pronounced in the Larne population, where turbary rights (the right of the individual to cut peat for domestic use) have been in place for almost 600 years, resulting in the loss of vast areas of peatland (Foss and O'Connell 1996). Ecological studies have also shown that peat cutting has a negative effect on the occurrence of *P. commune* populations. Cooper *et al.* (2001) found *P. commune* at a greater frequency (50%) in uncut quadrats compared with cut quadrats (44%). The findings of the present study suggest that fragmentation of bryophyte populations, even in a species with relatively effective spore dispersal mechanisms, has led to changes in the levels and partitioning of genetic diversity. With more peatland now being threatened by the impact of large-scale, mechanised peat cutting, these results highlight the need for careful management practices of these unique and vulnerable habitats.

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Table 1. *Description of* Polytrichum commune *populations analysed in this study with sample numbers*

Site	Description	Cut / Uncut	Subsample	**N**
Larne	Upland blanket bog, which has been extensively hand cut for centuries up to the present day.	Cut	1	10
			2	12
			3	6
			4	11
			Total	42
Slievanorra	Upland blanket bog, a large area of which is designated as a Nature Reserve and is protected from peat cutting and grazing. The remainder has been hand cut extensively.	Mixed	1	12
			2	8
			3	11
			4	7
			Total	38
Peatland's Park	Designated as an ASSI (Area of Special Scientific Interest). Large areas of intact lowland raised bog but some areas have been extensively drained and cut in the early 20th C, resulting in a mosaic of peat cuttings.	Mixed	1	13
			2	14
			3	30
			4	8
			5	14
			Total	81
Sperrins	Area of intact upland blanket bog.	Uncut	1	23
			2	5
			3	16
			Total	44
			TOTAL	200

Table 2. *Within-population diversity values at three microsatellite loci in* Polytrichum commune

Population	H Diversity			
	F-11	F-17	F-21	Average
Larne-1	0.889	0.956	0.844	0.896
Larne-2	0.742	0.788	0.455	0.662
Larne-3	0.800	0.600	0.600	0.667
Larne-4	0.746	0.746	0.582	0.691
Average	0.749	0.772	0.622	0.729
Slievanorra-1	0.742	0.924	0.939	0.869
Slievanorra-2	0.893	0.857	0.786	0.845
Slievanorra-3	0.946	0.911	0.782	0.879
Slievanorra-4	0.810	0.714	0.714	0.746
Average	0.846	0.852	0.805	0.835
Peatlands Park-1	0.974	0.848	0.744	0.855
Peatlands Park-2	0.934	0.923	0.780	0.879
Peatlands Park-3	0.917	0.906	0.729	0.851
Peatlands Park-4	0.893	0.964	0.607	0.821
Peatlands Park-5	0.747	0.846	0.846	0.813
Average	0.893	0.898	0.741	0.844
Sperrins-1	0.870	0.893	0.775	0.846
Sperrins-2	0.900	0.900	0.900	0.900
Sperrins-3	0.925	0.917	0.842	0.894
Average	0.898	0.903	0.839	0.880
Overall average	0.847	0.856	0.752	0.818

Table 3. *Analysis of molecular variance (AMOVA)*

Source of Variation	d.f.	Sum of squares	Variation		*P*
			Variance	%	
Among populations	3	1.977	-0.00098	-0.20	$P = 0.399$
Among subpopulations	14	9.152	0.01602	3.21	$P < 0.001$
Within subpopulations	182	90.578	0.48438	96.99	$P < 0.001$
Total	199	101.702	0.49942		

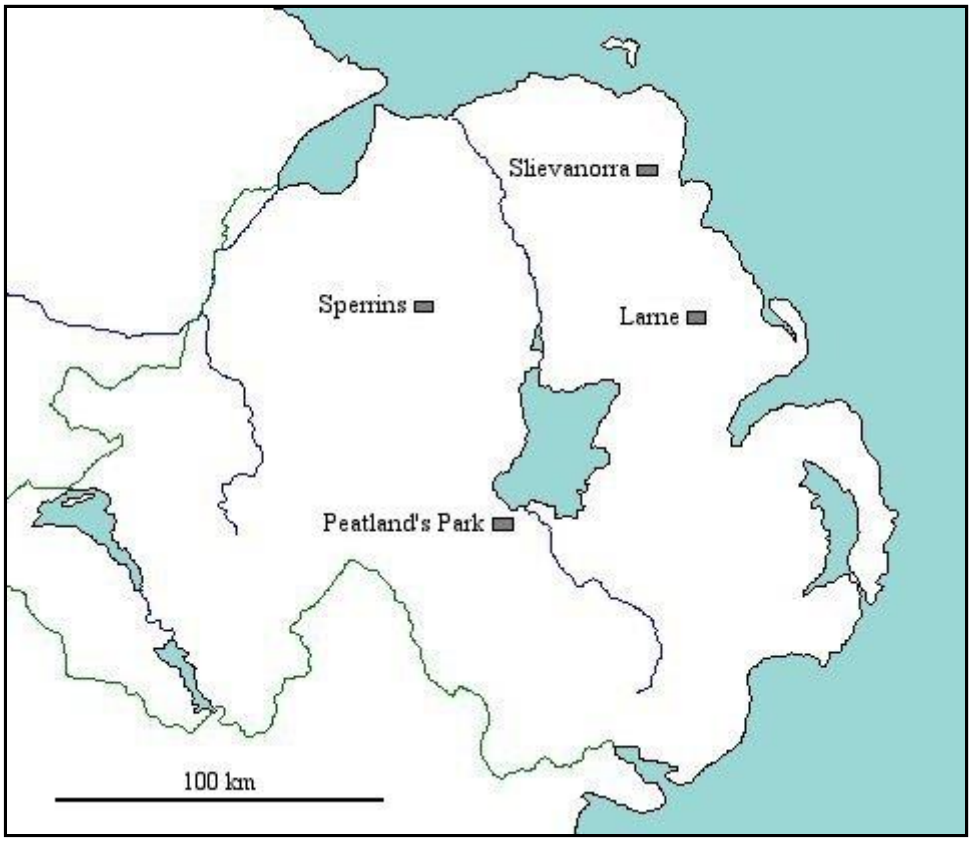
Table 4. *Partitioning of genetic variation between subpopulations within individual populations*

Population	% Variation among subpopulations	
Larne	13.53%	***
Slievanorra	1.19%	*
Peatland's Park	0.43%	*
Sperrins	-0.18%	NS

*** $P < 0.001$
* $P < 0.05$
NS Non-significant

Figure Legend

Figure 1. Map showing the location of populations of *Polytrichum commune* analysed in this study.


Slievanorra
Sperrins
Larne
Peatland's Park
100 km

Appendix. *Allele frequencies at three microsatellite loci in* Polytrichum commune *populations studied. Most common allele at each locus in each subpopulation is shown in bold.*

Locus	Allele	Larne				Slievanorra				Peatland's Park					Sperrins		
	(bp)	1	2	3	4	1	2	3	4	1	2	3	4	5	1	2	3
F-11	124	-	-	-	-	-	-	-	-	0.0769	-	0.0333	-	-	-	-	-
	126	-	-	-	-	-	-	-	-	0.0769	-	-	-	-	-	-	-
	128	-	-	-	-	-	-	-	-	0.0769	-	0.0667	-	-	-	-	-
	130	-	-	-	-	-	-	0.0909	-	-	-	0.0333	-	-	-	-	-
	132	-	0.0833	-	0.2727	0.0833	0.1250	-	**0.4286**	0.0769	0.1423	0.0333	0.1250	0.1429	-	-	0.1250
	134	-	-	-	**0.4545**	-	-	0.0909	-	0.0769		-	-	0.0714	-	0.2000	0.1250
	136	**0.3000**	0.0833	0.1667	-	**0.5000**	**0.2500**	**0.1818**	-	-	**0.2143**	0.1667	0.1250	0.1429	-	-	0.0625
	138	-	-	**0.5000**	-	-	**0.2500**	0.0909	-	**0.1538**		0.1000	0.1250	-	0.2173	**0.4000**	0.1250
	140	-	0.1667	0.1667	0.0909	0.1667	-	-	-	-	0.0714	0.0333	0.1250	-	0.1739	0.2000	**0.1875**
	142	-	-	-	-	-	-	-	-	0.0769	0.1423	-	**0.3750**	**0.5000**	-	-	0.0625
	144	0.1000	**0.5000**	-	0.1818	0.1667	**0.2500**	0.0909	0.2857	-	0.0714	0.0333	-	-	0.0434	-	-
	146	-	-	-	-	-	-	**0.1818**	-	-	-	-	0.1250	0.0714	**0.2609**	-	**0.1875**
	148	0.2000	-	-	-	-	-	-	-	-	-	**0.2000**	-		0.0434	0.2000	0.0625
	150	0.2000	-	-	-	-	0.1250	**0.1818**	0.1426	-	0.0714	0.1000	-	0.0714	0.0870	-	-
	152	0.1000	-	-	-	0.0833	-	-	-	0.0769	-	0.1000	-	-	0.0870	-	-
	154	-	-	-	--	-	-	-	-	-	0.0714	0.0667	-	-	0.0434	-	-
	156	-	-	-	-	-	-	-	-	0.0769	0.1423	0.0333	-	-	-	-	-
	158	-	0.1667	0.1667	-	-	-	0.0909	0.1426	**0.1538**	-	-	-	-	-	-	-
	160	0.1000	-	-	-	-	-	-	-	-	-	-	-	-	0.0434	-	0.0625
	170	-	-	-	-	-	-	-	-	0.0769	0.0714	-	-	-	-	-	-
F-17	144	-	-	-	-	0.0833	0.1250	-	-	-	0.0714	0.0333	-	0.3571	0.0870	-	0.0625
	146	-	0.1667	0.1667	0.0909	-	-	-	-	-	-	0.0333	-	0.0714	0.0434	-	-
	148	-	-	**0.6667**	-	**0.1667**	-	-	-	0.2308	-	-	**0.2500**	-	0.0870	-	0.1250
	150	-	-	-	**0.4545**	-	-	-	0.1429	0.2308	0.0714	0.0333	0.1250	**0.2143**	-	**0.4000**	0.0625
	152	0.1000	**0.4167**	-	0.2727	**0.1667**	**0.3750**	**0.3636**	**0.5714**	**0.3077**	**0.2143**	**0.2000**	-	-	**0.3043**	-	0.0625
	154	**0.2000**	0.2500	-	-	-	0.1250	-	0.1429	-	-	0.0333	-	-	0.0434	-	-
	156	-	0.0833	0.1667	-	**0.1667**	-	0.0909	0.1429	-	**0.2143**	0.1667	0.1250	-	0.0434	0.2000	**0.1875**
	158	0.1000	-	-	-	**0.1667**	-	0.1818	-	-	-	0.0667	-	0.1429	0.1304	-	**0.1875**
	160	**0.2000**	-	-	-	0.0833	-	-	-	-	-	-	-	0.0714	0.0434	-	0.0625
	162	0.1000	-	-	-	-	0.1250	0.0909	-	-	0.0714	0.0333	-	-	0.0870	-	-
	164	-	-	-	-	-	-	0.0909	-	-	-	0.0333	-	-	-	0.2000	-
	166	-	-	-	-	-	0.2500	-	-	-	-	-	0.1250	0.0714	0.0434	0.2000	-
	168	0.1000	-	-	-	**0.1667**	-	0.0909	-	0.1539	0.1429	0.0333	-	-	-	-	-
	170	0.1000	-	-	0.1818	-	-	-	-	-	0.0714	**0.2000**	0.1250	-	0.0434	-	**0.1875**
	172	-	-	-	-	-	-	-	-	0.0769	0.0714	-	-	0.0714	-	-	-
	176	-	-	-	-	-	-	0.0909	-	-	0.0714	-	0.1250	-	-	-	-
	178	0.1000	-	-	-	-	-	-	-	-	-	0.0333	0.1250	-	0.0434	-	-
	180	-	0.0833	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	184	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0625

Appendix (continued)

Locus	Allele (bp)	Larne				Slievanorra				Peatland's Park					Sperrins		
		1	2	3	4	1	2	3	4	1	2	3	4	5	1	2	3
F-21	134	-	-	-	-	0.0833	-	-	-	-	-	-	-	-	-	-	-
	140	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1875
	142	-	-	**0.6667**	-	**0.1667**	0.1250	0.1818	-	0.1538	0.1429	0.1000	**0.6250**	**0.2857**	0.0870	-	0.0625
	146	0.1000	0.0833	-	-	**0.1667**	0.1250	0.1818	-	0.1538	-	-	-	-	-	-	-
	148	-	-	-	-	0.0833	-	-	-	-	-	-	-	-	-	-	-
	150	0.2000	0.0833	-	0.1818	-	0.1250	-	0.1529	-	0.0714	-	0.1250	0.0714	-	-	0.1875
	152	0.1000	**0.7500**	0.1667	0.1818	**0.1667**	**0.5000**	**0.4545**	0.1429	**0.4615**	0.1423	0.2667	-	0.1423	0.1739	-	**0.3125**
	154	**0.3000**	0.0833	0.1667	**0.6363**	0.0833	-	0.0909	**0.5714**	0.2308	0.2143	**0.4000**	0.2500	**0.2857**	**0.3478**	**0.4000**	0.1875
	156	**0.3000**		-	-	**0.1667**	0.1250	-	0.1429	-	**0.4285**	0.2333	-	0.0714	0.3043	0.2000	0.0625
	160	-	-	-	-	0.0833	-	0.0909	-	-	-	-	-	-	-	0.2000	-
	166	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2000	-
	170	-	-	-	-	-	-	-	-	-	-	-	-	0.1429	0.0870	-	-