The population genetics of the Mute Swan *Cygnus olor* in Ireland

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Mute Swan population genetics were investigated from four localities throughout Ireland using protein electrophoresis. The products of ten presumptive loci were assayed in red blood cells and one in blood plasma. All but the latter (plasma esterase) were monomorphic. Allele frequencies at the EST locus did not differ significantly from previously published values from England and Scotland nor was there a significant spatial or temporal heterogeneity apparent within Ireland. These results do not support the hypothesis of reproductive isolation of Irish swan populations from England and Scotland or within Ireland. The previously recorded presence of limited variability in the English and Scottish Mute Swan populations at one locus (Lactate dehydrogenase) compared to those assayed in Ireland (where it was monomorphic) may mean that introduction(s) involved a limited number of individuals.

The importance of biochemical genetics in the understanding of population ecology and dynamics is well described (Lewtonin 1972, Berry 1977). The population genetics of the Mute Swan *Cygnus olor* has been investigated in England and Scotland by Bacon (1980a, 1981), who stated that a greater knowledge of Mute Swan genetics might be useful in the understanding of population dynamics (Bacon 1980b). Such studies have since elucidated some aspects of Mute Swan breeding biology (Birkhead *et al.* 1983, Bacon & Andersen-Harild 1987, Walter *et al.* 1991).

In Ireland, the Mute Swan is widely distributed with population estimates varying between five to seven thousand individuals (Monval & Pirot 1989). Two recent studies (Collins 1991, Smiddy & O'Halloran 1991) give details of the breeding biology of Irish Mute Swans. Movements between Ireland and Britain in general appear rare. Only one Mute Swan ringed in Ireland has been recovered in Wales (Anglesey) and one ringed in England recovered in Ireland (Cork). Thus it has been suggested by Smiddy & O'Halloran (1991) that the two populations may be considered genetically separate. However, a high resighting rate of Outer Hebridian Mute Swans on the northern coast of Ireland (14 birds colour-ringed in the Western Isles, Scotland have been resighted on the north coast of Ireland (Hutchinson 1989, Spray 1981)), suggests that mixing of the populations may be greater than thought. These sightings seem to be the exception to the observed patterns of movement, given the few recoveries from the many other studies of swans in England (Birkhead & Perrins 1986). The Irish Mute Swan appears to be sedentary, though very little is known about swan movements in Ireland. O'Halloran & Collins (1985) in a preliminary analysis of swan recoveries in Ireland have reported that 90% (n = 61) of ringed birds travelled less than 65 km and no adult travelled this distance. This is similar to movements observed in England (Ogilvie 1967).

Unlike the situation in England, Scotland and Wales (Ticehurst 1957), little is known historically about the Irish Mute Swan, though introduced birds were known to be present in Cork in the early 1700s (Smith 1750). Thompson (1851) reported the species from around Belfast and stated that it was not known in the wild state, but only in collections, and that he was uncertain of the date of its

introduction. All authors in the present century followed Thompson (1851) in regarding the species as an introduction although Collins & Whelan (1990) questioned this. They stated that the spread of the Mute Swan in the 19th and 20th centuries could be as a result of the decline in persecution rather than the spread of the species following an introduction. This has happened following protection in Denmark since *c.*1930, and Poland and the USSR since 1945. In Denmark populations have grown exponentially, doubling in the last 60 years (Bloch 1971, Wieloch 1991). Similar population increases have been observed in introduced swan populations in North America.

Protein electrophoresis provides a useful measure of genetic variability and composition (by the study of allelic distribution of polymorphic loci) (Ferguson 1980). This approach has been used by Bacon (1980a, 1981) in his studies of Mute Swan population genetics in England and Scotland, but not with Irish populations. This study set out to determine the level of genetic variability in Irish Mute Swan populations using protein electrophoresis and possibly to provide evidence on the scale of introduction of Mute Swans to Ireland. We describe the genetic variability of the Irish Mute Swan population as shown in particular for the flock at Cork Lough, and compare the variability to other sites within Ireland and with sites in Britain (Bacon 1980a, 1981).

Material and methods

Sample collection

Mute Swans were captured, under

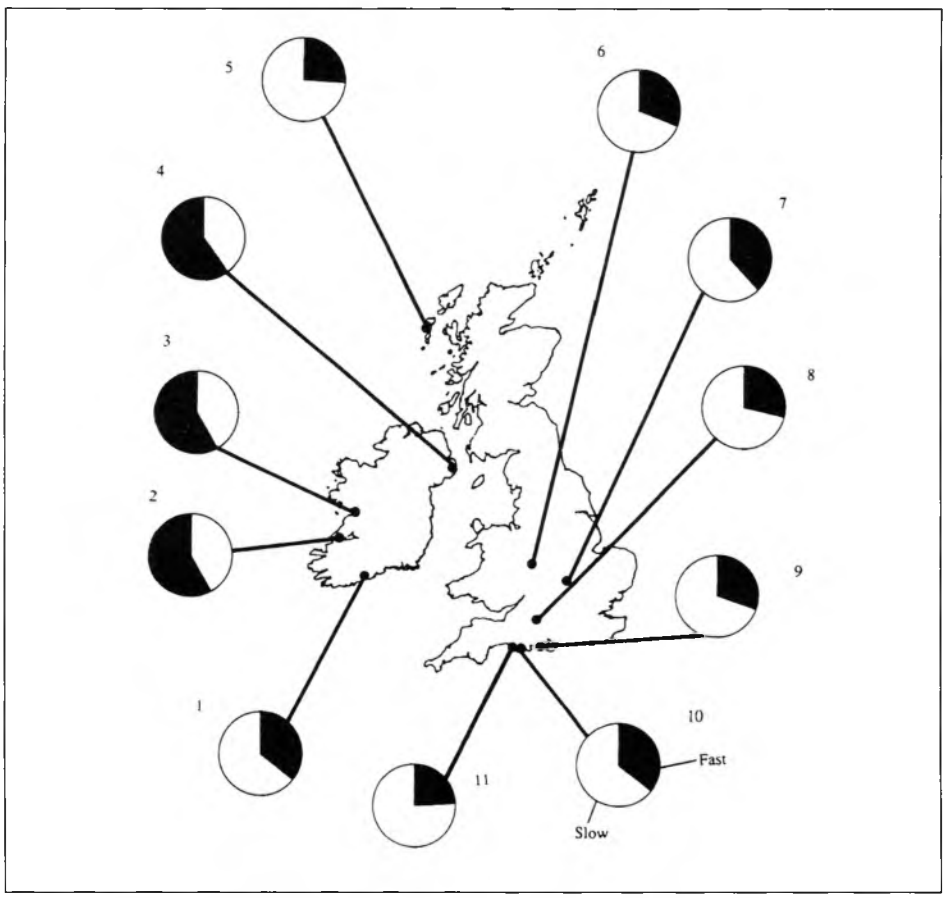

Figure 1. Location of sampling sites for Mute Swans and the pattern of esterase gene frequencies in Ireland and Britain; numbers refer to locations named in Table 2. (Data for Britain recalculated from Bacon 1981.)

licence, at four sites (Fig. 1 and Tables 2 and 3) during the winter of 1986 (Belfast and Cork Lough) and 1990 (Cork Lough, Limerick and Galway). The data refer to samples of swans caught from wintering flocks. Such flocks comprise juveniles, non-breeders and failed breeders from the surrounding area. Family data, i.e. related individuals whose genotypes do not therefore constitute an independent sample, were excluded in determining population genotype and allele frequencies. Similar procedures were followed by Bacon (1980a, 1981) and Bacon & Andersen-Harild (1987). Following age and sex determination, 3 to 5 ml of blood were drawn from the brachial vein using a 21 gauge needle. The blood was transferred to a 5 ml tube coated with dried lithium heparin and returned to the laboratory on ice.

Sample preparation and gel running

Blood samples were spun in a centrifuge for 10 minutes at 2000 x g, the plasma removed and the packed red blood cells were suspended and washed in 10 volumes of 0.9% of NaCl and shaken, re-centrifuged and the supernatant removed. The red cells were then lysed using an equal volume of 0.01% Triton X-100 in 0.9% saline solution, re-suspended, vigorously shaken and re-centrifuged. An aliquot of the supernatant was then removed and stored at -20°C until required for electrophoresis.

Starch gels (11% from Sigma Chemical Company, St Louis, MO) were prepared using Perspex moulds measuring 220 x 180 x 6 mm and two buffers (a) New (Ayala *et al.* 1972) and (b) TCB (Taggart *et al.* 1981). A sample of red cell lysate and/or plasma was absorbed on to a 2 mm x 6 mm piece of Whatman No. 3 filter paper for application to gel. The gels were run at 150-300 V DC for 4-6 hr at 4°C, with additional cooling using bags of crushed ice. Staining followed Ayala *et al.* (1972) or Harris & Hopkins (1976) with minor modifications. Bands were recorded by tracing directly from the gels.

Table 1. Details of enzymes of Mute Swans assayed, showing Enzyme Commission (EC) number and putative enzyme loci. Optimum tissue and buffer combinations and number of swans sampled, are given for each locus. (*AAT*-1* and *MDH*-1* were too weakly expressed to be typed reliably.)

Enzyme	EC No.	Locus	Buffer	Tissue	*n*
Asparate aminotransferase	2.6.1.1	*AAT*-2*	New/TCB	RBC	20
Esterase	1.1.1.1	*EST**	TCB	Serum	65
Glucosephospate isomerase	5.3.1.9	*GPI**	TCB	RBC	46
Haemoglobin	NA	*Hb**	New	RBC	52
Isocitrate dehydrogenase	1.1.1.42	*IDHP**	New	RBC	20
Lactate dehydrogenase	1.1.1.27	*LDH*-1*	New	RBC	46
		LDH-2*	New	RBC	46
Malate dehydrogenase	1.1.1.37	*MDH*-2*	New	RBC	52
Phosoglucomutase	5.4.2.2	*PGM**	TCB	RBC	10
Phosogluconate dehydrogenase	1.1.1.44	*PGDH*	New	RBC	20
Superoxide dismutase	1.15.1.1	*SOD**	TCB	RBC	20

Table 2. Esterase genotype frequencies for Mute Swans in Ireland (the present study) and England and Scotland (Bacon 1981). *P* is for X^2 tests with 1df for deviation from Hardy-Weinberg expectations (see text).

		Genotype frequency					
Location	No.[d]	FF	FS	SS	*n*	*P*	Reference
Cork Lough[a]	1	20	25	5	50	NS	This study
Cork Lough[b]	1	5	9	8	22	NS	This study
West of Ireland	2/3	4	6	2	12	NS	This study
Belfast	4	1	6	3	10	NS	This study
Outer Hebrides	5	3	15	22	40	NS	(Bacon 1981)[c]
Midlands	6	12	52	57	121	NS	(Bacon 1981)[c]
Oxford	7	5	18	15	38	NS	(Bacon 1981)[c]
Salisbury	8	2	10	14	26	NS	(Bacon 1981)[c]
Christchurch	9	13	56	63	132	NS	(Bacon 1981)[c]
Weymouth	10	3	10	8	21	NS	(Bacon 1981)[c]
Abbotsbury	11	6	38	59	103	NS	(Bacon 1981)[c]

[a]Cork Lough 1990, [b]Cork Lough 1986, [c]recalculated from Bacon (1981),
[d]Site number in Figure 1.

Results

The nine enzymes and one non-enzymatic protein examined were presumed to be determined by 11 loci (LDH was coded for by two loci) (Table 1). Eight enzymes occurred in red blood cells (RBC) and one in plasma (see Table 1 for the appropriate buffer or tissue combinations used). All proteins listed in Table 1 except the plasma esterase (EST), were monomorphic in Mute Swans at all four locations investigated in Ireland (Fig. 1, Tables 2 and 3). The banding pattern for the esterase locus on a TCB buffer system is shown in Figure 2.

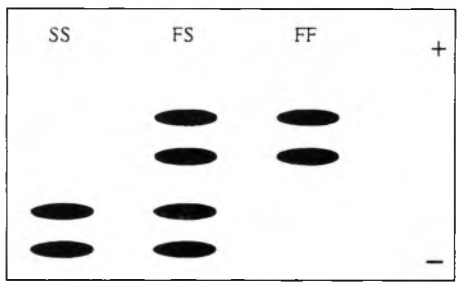

Figure 2. Zymogram of the esterase banding pattern of Mute Swans observed on a TCB buffer system.

The genotype frequencies and allele frequencies at the diallelic esterase locus are given in Tables 2 and 3 and Figure 1 which also include English and Scottish allele frequencies recalculated from Bacon (1980a). The genotypic frequencies did not differ significantly from the Hardy-Weinberg prediction using a Chi-squared test with one degree of freedom, in cases where numbers were adequate for testing (i.e. expected frequencies >5) (Table 2). The cygnets from a family party from Limerick were not included in the final genotype and allele frequency analyses (all other individuals appeared unrelated and were thus included). This single family party displayed the following inheritance ratio for the esterase locus: parents, both FS; cygnets, one SS, two FS.

A number of comparisons of plasma esterase allele frequencies (Table 3) were made using Chi-squared test. There was no difference (X^2_1 = 0.146, N.S.) between the allele frequencies at the esterase locus for the Cork Lough samples of 1986 and 1990 (Table 3) (all samples from known individuals). Thus the two samples were pooled (Cork Lough[c], Table 3). There was no significant difference in the pattern for the esterase allele frequency between locations in Ireland (X^2_3 = 3.59, N.S.) or England and Scotland (X^2_6 = 3.73, N.S.). The sample data from Limerick and Galway were pooled as the expected values at these sites were less than five thus invalidating the requirements of the X^2 test. There was no significant difference (X^2_{10} = 13.49, N.S.) when samples from all locations were pooled, though the West of Ireland, Belfast and Abbotsbury showed most deviation from the average frequencies. Finally there was no significant heterogeneity (X^2_1 = 3.46, N.S.) when the pooled data from each country were compared.

Discussion

In the present study we found that nine enzyme loci and the single non-enzymatic protein locus (haemoglobin) examined in

Table 3. Esterase allele frequencies for Mute Swans in Ireland with data from England and Scotland (Bacon 1981) included for comparison. (See Fig. 1.)

Location	Allele Frequency No.[d]	F	S	*n*	Reference
Cork Lough[a]	1	0.35	0.65	50	This study
Cork Lough[b]	1	0.43	0.57	22	This study
Cork Lough[c]	1	0.37	0.63	72	This study
Limerick	2	0.58	0.42	6	This study
Galway	3	0.58	0.42	6	This study
Belfast	4	0.60	0.40	10	This study
Outer Hebrides	5	0.26	0.74	40	Bacon (1981)[c]
Midlands	6	0.31	0.69	121	Bacon (1981)[c]
Oxford	7	0.38	0.62	38	Bacon (1981)[c]
Salisbury	8	0.29	0.71	26	Bacon (1981)[c]
Christchurch	9	0.31	0.69	132	Bacon (1981)[c]
Weymouth	10	0.36	0.64	21	Bacon (1981)[c]
Abbotsbury	11	0.24	0.76	103	Bacon (1981)[c]

Superscripts as in Table 2.

the red blood cells of the Mute Swans were monomorphic at all locations sampled in Ireland. Bacon (1980a) also found most RBC loci to be invariant, but reported one lactate dehydrogenase (LDH) locus to be variable at low frequency at most English and Scottish sites. Exceptions were areas of colonial nesting (e.g. Abbotsbury and Weymouth) where the frequency of the heterozygote LDH phenotype was higher (0.152 and 0.166 respectively) and this is also true in Denmark (Bacon & Andersen-Harild 1987) for colonial Mute Swans. In Ireland the Mute Swan is generally a solitary breeder, but two colonial breeding sites have recently been reported (Hutchinson 1989). However, these sites were not included in the present study. The absence of polymorphism at LDH found in the present study could be due to the fact that it is not present in the Irish population. This could be due to the founder effect, i.e. the Irish population was founded by colonizers whose genetic composition consisted of a subset of that of the population of origin, which lacked the alternative *LDH** allele. Interestingly, Bacon (1980a, 1981) failed to detect this polymorphism in another introduced island population, the Outer Hebrides. However, the alternate allele may be present at such a low level in the Irish population that our sample sizes were insufficient to detect it. If this is so, then investigation of swans from colonial breeding sites may be fruitful (see above).

Plasma esterase was the only enzyme shown to be polymorphic in Ireland (Fig. 2). Because we used a different buffer system to Bacon (1981) the appearance of the phenotype was not the same, but factors discussed below confirm that we were considering the same system. The gene frequencies at the esterase locus for the Cork Lough swans showed no significant temporal variation (Table 2). Bacon (1980a) found a similar absence of temporal variation for his three year study at Oxford. The fact that similar allele frequencies were found for Cork Lough swans in 1990 (analysed in Cork) and 1986 (analysed in Oxford) permits the intercalibration between the results of this study and those of Bacon (1980a, 1981) (also obtained in the Oxford laboratory). Thus, as indicated above, allele frequencies from Bacon (1981) were included in Figure 1 and Table 2. Furthermore, segregation ratios from a breeding pair and three cygnets illustrated simple Mendelian inheritance, demonstrating that a single diallelic locus codes for the enzyme, as has been shown previously by Bacon (1980a). From a parental cross involving two heterozygotes the expected ratio of esterase genotypes, SS:FS:FF, would be 1:2:1. The observed genotype ratio of 1:2:0 from three offspring does not deviate substantially from this pattern of inheritance (see Results.)

No significant heterogeneity was found in allele frequencies at *EST** between the four Irish locations (though the small number of swans sampled in areas other than Cork Lough should be noted). One interpretation of this finding is there may be sufficient movements of individuals between the different areas to maintain genetic homogeneity (Fig. 1). Very little is known about swan movements in Ireland. The main reason is a low level of swan ringing and as a consequence, a low level of recoveries. O'Halloran & Collins (1985) in a preliminary analysis of a small number of Mute Swan ringing recoveries (n = 61) showed that approximately 7% of swans travelled over 100 km. A recent colour ringing study, with several thousands of sightings, has shown that patterns of Mute Swan movements are along river valleys and to and along the coast, with some individuals travelling linear distances of 250 km (O'Halloran unpublished for Ireland, Ogilvie 1967 for England, Scotland and Wales). Though these latter movements appear to be less common it is conceivable, given that Ireland is a small island, that sufficient mixing of genes exists to prevent the development of population structure.

Perrins (1991) working with a population of swans which was almost entirely ringed, achieved a recovery rate of only one in four birds. Considering the much smaller numbers of birds ringed in Ireland, the scarcity of recoveries and hence recorded movements need not mean that such movements do not occur. The similarities in the esterase gene frequencies both within Ireland and between Britain and Ireland suggests that there is indeed much more mixing of the population than recorded by colour-ring resightings or recoveries. It must be emphasised however, that this result is based on a single locus and may

not reflect the actual situation in the genome as a whole. On the other hand, if isolation of these populations is real, sufficient genetic differences may not have arisen by selection or drift to be identified by these techniques.

Based upon the proteins examined in this study, there appears to be no significant genetic difference between Mute Swan flocks within Ireland or between those in Britain and Ireland. There is an observable difference in that an LDH locus which is weakly variable in English populations, was invariant in the Irish populations we investigated. However, such a small difference could not be tested statistically. The genetic similarity of the EST allele frequencies of Mute Swans in England and Scotland suggest that the Irish Mute Swan probably arrived from that country, though whether by anthropogenic introduction or natural colonisation is of course impossible to say. Nor can the size of the introduction be estimated. The lack of LDH polymorphism in Ireland demonstrated by this study suggests that the introductions were small. On the other hand the presence of the esterase polymorphism in Ireland with similar allele frequencies to Britain suggests fairly substantial introductions unless balancing selection is operating. Since the variation in allozymes is so low it is suggested that mini-satellite DNA analysis (Burke 1989) for example, might show more variation and thus would be more useful in addressing problems of Mute Swan population structure.

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