The role of genetics in conserving small populations

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Section 3 – Genetics and species recovery

With the data provided by genetic techniques, such as those discussed in the previous section, it should be possible to start to take account of genetic variation in conservation management. Species Recovery or Action Programmes are increasingly used by national and international organisations as a framework for the long-term conservation management of threatened species.

One of Britain's best known reintroduction projects has been that of the red kite. Pedro Cordero and his colleagues illustrate the value of genetic fingerprinting for monitoring the introduced populations. DNA fingerprinting has allowed conservationists directly to confirm parentage and also to assess the survival and breeding success of released birds. Introduced populations are genetically more variable than their remnant native counterparts, and their reproductive success is higher. However, the effects of genetics and environment are still to be fully unravelled.

Some of the problems and challenges in the conservation of the world's rarest seal, the Mediterranean monk seal, are described by Helen Stanley and John Harwood. Analysis of mitochondrial DNA suggests limited gene flow between the two remaining breeding populations (separated by 4500 km). The evolutionary divergence between the Mediterranean monk seal and the Hawaiian monk seal is greater than was suspected. Moving individuals between the two remaining Mediterranean monk seal populations may be possible, but is not currently recommended.

David Balharry and his co-workers introduce the complex situation regarding the conservation of one of Britain's rarest mammals – the wildcat. New genetic techniques have tended to complicate, rather than simplify, the problem over wildcat identification. It is not even clear whether the wildcat is a taxon distinct from the domestic cat. If it is not, is there anything that we should be trying to conserve? The wildcat example raises many other nature conservation issues, such as the cultural importance of species and wildlife law enforcement, and reminds us that although the study of genetics can be an important part of nature conservation management, it cannot provide all the answers.

One of Britain's rarest trees is the Plymouth pear. Andy Jackson and his co-authors explain how molecular genetic techniques such as Randomly Amplified Polymorphic DNA (RAPD) fingerprinting have been crucial in underpinning the conservation of the two remaining, but isolated, populations. Although short-term conservation hinges on protection of individual specimens, long-term conservation is more likely to be dictated by genetic factors.

The section is completed by Mike Maunder and Alistair Culham, who offer a general account of the work and value of botanical garden collections in the *ex-situ* conservation of rare plant species. They use international case studies to illustrate the potential benefits of the (largely historical) human passion for collecting and cultivating rare and attractive plants. The future value of botanical collections for conservation looks promising, as new techniques such as genetic screening are incorporated into multidisciplinary international programmes.

The papers in this section therefore illustrate the wide range of uses for, and applications of, genetic techniques in the conservation management of small populations. These currently range from tools for monitoring the success of reintroductions to the long-term planning of future recovery programmes.

Studies of the genetics of a naturalised population of red kites *Milvus milvus* in England established by translocation

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The red kite *Milvus milvus* populations of England and Scotland became extinct over a hundred years ago. In 1989 to 1994, red kites were translocated from Spain, Sweden and Wales and released into selected areas of England and Scotland in an attempt to re-establish breeding populations. The genetics of the red kites released and naturally-reared in England were monitored along with their fate and breeding success during this period. This paper describes the results of this monitoring, and shows that the molecular recognition of founding individuals can be a valuable adjunct to any re-establishment policy. Apparent variation in the performance of birds from different provenances suggests that the genetic architecture of the stock should be borne in mind when selecting individuals for such an exercise.

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Introduction

The red kite *Milvus milvus* was formerly widespread over much of Europe, western Asia and North Africa, although persecution has led to a dramatic decrease in range and abundance during the last 300–400 years. In Britain, it used to be familiar and abundant, and was even protected in the Middle Ages because of its useful habit of scavenging the streets and removing refuse. However, changes in legislation (the Tudor vermin acts) stripped the red kite of its protection in 1566, and promoted its persecution in England and Wales. Similar changes had occurred in Scotland under a decree from James II in 1457 (Lovegrove 1990). Later, persecution consequent upon the establishment of hunting estates, and the Victorian passion for collecting eggs and skins, led to the collapse of the British red kite population during the 18th and 19th centuries.

Between 1890 and 1990, red kites bred only sporadically in England and Scotland, while in Wales nests continued to be robbed annually and birds were shot and poisoned. As a result of this pressure, the British red kite population was driven to the edge of extinction during the 1930s (Davis 1993). A vigorous campaign of protection together with a more enlightened attitude to red kite conservation in Wales, especially by farmers, has encouraged the population to increase, albeit very slowly, from its all-time low in the 1930s to over 120 pairs in 1995. Despite this success, the red kite is still very rare and vulnerable in British terms.

It has been suggested (Evans & Pienkowski 1991) that there are three reasons why the red kite population continues to recover only slowly in Wales. First, breeding productivity is low compared with other parts of

Europe (Cramp & Simmons 1980). Whether this is because the habitats of central Wales are marginal for this species, or inbreeding depression following a loss of genetic variability through the bottlenecking of the population (or both) remains unresolved. Second, illegal killing of red kites continues (Cadbury 1992). Third, red kites are very philopatric (Newton, Davis & Davis 1989) and tend to breed close to their natal areas. In view of the distance between Wales and the larger continental populations, the British red kite population cannot rely on the immigration of continental birds to increase population size.

The red kite thus fell to very low numbers early in the 20th century, and there were real fears for its continued survival as a British bird (Davis 1993). During the 1980s, there was much discussion between nature conservation groups concerning the feasibility of re-establishing the red kite in parts of its previous range (reviewed by Evans & Pienkowski 1991). It was agreed that the species fulfilled internationally accepted IUCN criteria for re-establishment (Evans 1994), namely:

1. there is good historical evidence of former natural occurrence;
2. there is a clear understanding of why the species had been lost, being due to human agency, and there was little possibility of natural recolonisation;
3. the factors causing extinction have been rectified;
4. there is suitable habitat of sufficient extent to allow a self-perpetuating population to become established;
5. individuals are available from closely adjacent populations (Spain, Wales and Sweden);
6. the removal of young birds will not threaten the continued existence of the population from which they are removed.

In the light of this evaluation, a re-establishment project was undertaken from 1989 to 1994, under the auspices of the Nature Conservancy Council (NCC) (later the Joint Nature Conservation Committee (JNCC), the Countryside Council for Wales (CCW), English Nature (EN) and Scottish Natural Heritage (SNH)) and the Royal Society for the Protection of Birds (RSPB), to evaluate whether red kite population centres could be re-established in England and Scotland (Evans 1994; Evans *et al.* 1994). This paper reports on the genetic monitoring of the English operation which released a total of 93 red kites over a six year period from three source populations (Table 1).

Table 1. The number and origin of red kites released into southern England.

	1989	1990	1991	1992	1993	1994
Sweden	4	–	–	–	–	–
Wales	1	2	4	–	–	–
Spain	–	11	11	20	20	20
Total	5	13	15	20	20	20

Methods

As part of the monitoring, each bird was given a uniquely numbered ring and also a wing-tag was applied to assist with field identification (Evans & Orr-Ewing 1992). Blood samples of approximately 2 ml were taken from all individuals before they were released into the wild. When breeding took place, nests were located and the contents monitored. Most of the released birds retained their patagial tags for several years, so they could be identified at the nest. Their progeny were also ringed for subsequent identification, and a similar blood sample was taken before fledging. This procedure resulted in a series of blood samples from the released birds and those young reared to fledging in the new population. These were stored in EDTA tubes before molecular analysis.

DNA was extracted from each sample using standard protocols (Wetton & Parkin 1992). Three types of analysis were undertaken: matriline

determination and sexing; parentage assessment; and genetic variability of the populations. For the first, we used a sex-specific probe that has been shown to hybridise only with DNA from the female (May, Wetton & Parkin 1993). This probe reveals a multi-band profile that is invariably transmitted from mother to daughter, allowing an assessment of the female lineage (matriline). For parentage assessment, we used multilocus probes derived from red kites (May 1993; May *et al.* 1993) which reveal typical multi-band genetic fingerprints after hybridisation. The same probes were used, at a higher stringency washing, to reveal a single-locus profile. These were also used to confirm parentage, and for the estimation of genetic variation.

Full technical details of the restriction digests, electrophoresis conditions, and blotting and hybridisation protocols are given in the references cited above. The DNA studies were used to:

1. determine the sex of each bird;
2. confirm parentage at nests where the patagial tags of the adults were clearly seen;
3. establish parentage where the patagial tags were not read but identity was suspected from other visual clues;
4. establish parentage where adults had been identified clearly in previous (or subsequent) years but were missed in the year in question;
5. seek possible parents where an adult had not been seen associated with a particular nest;
6. examine the genetic variability of the introduced birds and compare this with the endemic Welsh population.

In a multilocus profile ('genetic fingerprint'), about half of the minisatellite bands can be traced back to the male parent, and about half to the female. These figures are only approximate, since they depend upon the number of bands that appear in the same place in unrelated individuals. Random individuals from an outbred species such as the house sparrow *Passer domesticus* possess few bands in common, and their profiles are very variable (Wetton & Parkin 1992). Other species, such as inbred populations of naked mole rats *Heterocephalus glaber* (Reeve *et al.* 1990), have much more similar profiles, and a correspondingly high proportion of shared bands. However, within a known population, a comparison of two profiles, and in particular an estimate of the proportion of bands that are shared, can give an indication of the relatedness of the individuals involved.

Typically, when two parents are compared with one of their progeny, all of the minisatellite bands in the offspring can be traced back to one or other adult. Any that cannot be traced back must be newly arisen mutations, and rarely are more than two of these found in a genuine trio of parents and offspring. This will be discussed in greater detail elsewhere (Evans, Cordero & Parkin in press), but more than two 'mismatching' bands in a red kite profile is good evidence that parentage has been wrongly assigned.

Results

Summary statistics of the introduced population are given in Table 2. Breeding was confirmed for the first time in 1991 when two pairs bred unsuccessfully. The first young were fledged into the wild in 1992. The number of breeding birds rose to 20 pairs in 1994, the last year of intensive observation, when 37 young fledged successfully.

Confirmation of parentage of English-reared birds

Table 3 shows the genetic resemblance between nestlings and their attendant adults at a nest site in 1993 and 1994. The same two adults were observed at the nest in each year, and four young

Table 2. Summary statistics of the red kite reintroduction into southern England.

	1989	1990	1991	1992	1993	1994
Number released	5	13	15	20	20	20
No. released surviving 12 months	1	6	14	15	15	20
No. of naturally fledged young	–	–	0	9	14	37
No. of these surviving 12 months	–	–	0	8	12	32
Red kites surviving in June 1995	0	5	12	21	26	52
No. of breeding pairs	–	–	2	4	9	20
Proportion that bred at least once during 1991–94	0%	46%	80%	79%	–	–

Table 3. Summary statistics of DNA profiles from two families of red kites reared by the male HT38631 and female HT38639 in successive years.

	Ring number	*Fingerprint bands*			*Bands of young shared with*		
	young	*male*	*female*	*young*	*male*	*female*	*neither*
1993	HT43985	11	10	9	7	5	0
	HT43986	11	10	12	10	6	0
1994	HT50121	11	10	10	8	5	0
	HT50122	11	10	11	8	5	0
	HT50123	11	10	12	8	8	0

were raised to fledging. The number of clearly identifiable bands in the fingerprint profile is shown for each individual, together with the number shared by each nestling with the adults. All of the bands identified in the nestlings could be traced back to the adult profiles, so there were no mis-matching bands, suggesting that the adults were indeed the parents.

Parentage was confirmed in all English nests at which both adults were seen. There was no evidence of mis-matching bands in the profiles that might indicate either extra-pair fertilisation or egg-dumping. We have also confirmed breeding by six red kites in 1994 that had themselves been reared in the wild. These are second generation birds, and probably the first red kites born of wild parents in England for over 100 years.

Distinguishing between two possible parents

DNA profiles can also be used to distinguish between two potential parents when rings or wing tags have not been read clearly. In 1994, a male (HT38637) was seen at a nest and successfully reared three young. The female was not seen clearly, and could have been either HT38633 or HT38645. When the DNA profiles of these three adults were compared with the nestlings, the results shown in Table 4 were obtained. Female HT38645 shares few bands with the nestlings, and several of their bands cannot be assigned to either her or the male. On the other hand, female HT38633 shows a perfect match with the nestlings. We conclude that the true biological parents of this brood were HT38637 and HT38633, and this was confirmed with single-locus probes (data not shown).

In every case where there was doubt over parentage, the DNA profiles were able to differentiate between possible parents.

Table 4. Summary statistics for distinguishing between two females seen in proximity to a brood of nestlings.

	Ring number			*Fingerprint bands*			*Bands shared with*		
	male	*female*	*young*	*male*	*female*	*young*	*male*	*female*	*neither*
1994	HT38637	HT38633	HT50130	12	14	13	7	8	0
	HT38637	HT38633	HT50131	12	14	13	10	5	0
	HT38637	HT38633	HT50132	12	14	12	8	7	0
1994	HT38637	HT38645	HT50130	12	11	13	7	1	5
	HT38637	HT38645	HT50131	12	11	13	10	1	3
	HT38637	HT38645	HT50132	12	11	12	8	1	4

Table 5. Comparative re-sighting figures from year n to year n+1 for red kites hatched from nests in Wales and England, and those released into southern England under the re-introduction programme. Totals given in parentheses. [1]From Newton, Davis & Davis (1989). [2]Released in 1989–1992. [3]Fledged in 1992.

Origin	No.	1st year		2nd year		3rd year	
Welsh natives[1]	70	60%	(42)	79%	(33)	94%	(31)
Welsh releases[2]	7	57%	(4)	75%	(3)	100%	(3)
Swedish releases[2]	4	25%	(1)	0%	(0)	0%	(0)
Spanish releases[2]	42	74%	(31)	94%	(29)	93%	(27)
English natives[3]	9	89%	(8)	100%	(8)	100%	(8)

Survival and breeding of the introduced birds.

The field observations allowed the estimation of the survival of English birds from year to year. Of 42 red kites from Spain released between 1989 and 1992, 74% were relocated at one year of age, 94% of these were located at two years, and 93% of these at three years. These figures are compared with similar data from native, wing-tagged birds in Wales in Table 5. The data differ significantly between locations in both first and second years. This is unlikely to be due to differences in dispersion because the English releases seem to range more widely that those in Wales (Evans unpublished data). It could be because of more intensive searching for the English birds, but could also reflect a higher survival of the introduced birds. There is little doubt that red kites of Spanish origin breed more successfully in England than the native British population does in Wales (Table 6). Brood sizes at the time of ringing are significantly larger among the released Spanish red kites ($p < 0.0001$). The sample sizes are small, so we have used Fisher's exact test, modified for larger contingency tables (Wells & King 1980). We also have proof of birds (of Spanish origin) breeding at one year of age in England (Evans, Cordero & Parkin in press), a phenomenon that is hitherto unrecorded in the species.

Table 6. Brood sizes of red kites at fledging among native Welsh birds and re-introduced birds in southern England. [1]From Davis & Newton (1981). [2]Between 1992 and 1994.

	Size of brood			
	1	2	3	Mean
Wales[1]	181	87	4	1.35
England[2]	8	11	10	2.07

Discussion

The reproductive success of the population in Wales is as low as any that has been recorded in red kites (Arcà 1989; Cramp & Simmons 1980). This could be ascribed to either ecological or genetic factors (or both). The red kite population in Wales does not live there by choice. It was reduced to a fragment, and survived in this region through a combination of dedicated protection and a lower level of persecution than elsewhere in Britain. The environment is harsh for the species, resulting in relatively poor food supplies, and feeding conditions that have a negative impact on fledging rates (Newton, Davis & Moss 1994). It was shown by May (1993) that a sub-population south of the main breeding centre in mid-Wales was genetically more variable at both matrilineal and autosomal loci, albeit still less variable than populations in Spain and Germany. This group of birds also had a higher reproductive success than the main population.

Our own results confirm those of May *et al.* (1993) which showed that the birds from Wales (and to a lesser extent, Sweden) are genetically depauperate compared with the larger populations in Spain and Germany. Table 7 shows an example of the results obtained by screening one nestling each from a series of nests in Spain, England and Wales

Table 7. Number of alleles and heterozygosity of released and native red kites at cMmi30. (* Released into England, but not yet breeding, and so not related to the English nestlings.) English nestlings and Spanish releases do not differ ($p > 0.5$), but they are significantly more heterozygous than the birds from Wales ($p << 0.001$).

	Alleles	Heterozygotes	Homozygote	Total
English nestlings	11	18	0	18
Spanish releases*	12	19	2	21
Wales 1993	5	32	26	58

with a single locus probe (cMmi30). The number of alleles and the heterozygosity are both significantly lower in the birds of Welsh origin, suggesting that they might be inbred, and thus liable to show inbreeding depression. Furthermore, only two matriline haplotypes were recorded in central Wales (May, Wetton & Parkin 1993), and we only found the same two among 60 broods hatched in Wales in 1993. Nine separate matrilines were identified among the 18 females breeding in England between 1991 and 1994, supporting the view that the translocated birds from Spain are genetically more variable.

Unfortunately, similarly inferior reproductive characteristics are displayed by avian species that inhabit ecologically inclement environments or show inbreeding depression. This gives rise to a confounding effect. The environment in Wales is harsh for red kites (Newton, Davis & Moss 1994), and the species may also suffer from inbreeding depression. More extensive European populations inhabit less hostile environments, and may not be inbred. In Wales, the intermediately variable southern sub-population is more successful than the main population, but less so than continental birds. It is not possible to learn anything from a direct comparison of these data. However, we can examine the survival and reproduction of birds from different populations released into England. This is difficult because of the low numbers but, nevertheless, some interesting indications are revealed.

Table 8. Survival to breeding of introduced red kites in England.

Origin	Number breeding (i.e. laying eggs)	Did not breed	Total	% breeding
Wales	3	4	7	43
Spain	30	12	42	71
Sweden	0	4	4	0
England	7	2	9	78

Table 8 shows the number of released birds from Spain, Sweden and Wales that bred successfully in southern England. The numbers in this table are a little small for analysis, but birds of Spanish origin were recruited into the population more (71%) than those of Welsh origin (43%). No bird of Swedish origin was observed breeding in England, and this observation was confirmed by the genetic profiling. The difference between the Spanish and Swedish data is significant ($p < 0.02$, Fisher's exact test (Sokal & Rohlf 1981)) and the Swedish population is also less polymorphic than the Spanish one (May 1993). Two males and one female of Welsh origin were recruited into the breeding population (Table 9). Both males bred for the first time at three years of age, which is significantly later than males of Spanish origin that bred at one or two years of age ($p < 0.01$, Fisher's exact test). The female from Wales bred for the first time at two years of age, while females of Spanish origin bred at one or two years old.

The sample sizes are all very small. Nevertheless, some differences are statistically significant, and a pattern begins to emerge. Compared with red kites of Spanish origin released into the same environment, birds of Welsh origin

Table 9. Age at first breeding among introduced red kites.

Origin	1 year	2 years	3 years	4 years	Total
Males from Wales	–	–	2	–	2
Males from Spain	4	11	–	–	15
Females from Wales	–	1	–	–	1
Females from Spain	5	10	–	–	15

survive less well, provide fewer recruits to the new population, and breed for the first time at an older age. They probably also rear fewer (certainly no more) young. Taken together, these might suggest *prima facie* that they are less successful. They are also less heterozygous as revealed by their minisatellite profiles. There is an increasing body of evidence suggesting that heterozygosity is important in determining individual and population fitness. A review by Allendorf & Leary (1986) concludes that components of fitness are correlated with heterozygosity in many species, and that this should be taken into consideration in any conservation programme.

Another re-establishment programme using continental red kites as release stock has recently been initiated in the East Midlands of England (Carter, Evans & Crockford 1995). Other release sites are planned to enable the breeding populations in England, Scotland and Wales to merge. This will provide a valuable opportunity to re-examine whether there are significant differences in breeding performance and survival between inbred (native) and more heterozygous (re-established) populations within comparable environments. If inbreeding depression is an important factor in reducing ecological fitness, then 'old school' conservation efforts that focus on making rare populations common again should take account of restoring their natural genetic variability and composition, alongside the aims of restoring their natural abundance and range.

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Genetic differentiation among subpopulations of the highly endangered Mediterranean monk seal

Helen F. Stanley and John Harwood

Stanley, H.F., & Harwood, J. 1997. Genetic differentiation among subpopulations of the highly endangered Mediterranean monk seal. *In*: *The role of genetics in conserving small populations*, ed. by T.E. Tew, T.J. Crawford, J.W. Spencer, D.P. Stevens, M.B. Usher & J. Warren, 97–101. Peterborough, JNCC.

The Mediterranean monk seal *Monachus monachus* is now restricted to a fraction of its former range and, with only 400–550 individuals, is the most threatened seal species in the world. There is unlikely to be any interchange of individuals between the two remaining breeding populations, which are 4500 km apart. We investigated genetic variation within and between *M. monachus* populations using mitochondrial DNA sequence analysis. We found small, but possibly fixed, differences in mtDNA between seals from the two populations. Our results suggest that there has been only limited gene flow between the two populations, but do not support designation of the Atlantic population as a distinct subspecies. The genetic data also suggest that genetic incompatibility is unlikely. A large divergence was found between *M. monachus* and the Hawaiian monk seal *M. schauinslandi* and the evolutionary divergence between these species is greater than was previously appreciated. We caution against using inter-specific generalisations in the development of a conservation programme for the highly endangered but poorly studied *M. monachus*.

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Introduction

Current and historical status of genus

The subfamily Monachinae contains the three most endangered seal species in the world. The Caribbean monk seal is probably extinct. The Hawaiian monk seal has a total population of 1200–1400 individuals (Marine Mammal Commission 1995) and has been severely reduced in numbers over the last 30 years (Gilmartin & Eberhardt 1995). The current population of the Mediterranean monk seal is estimated to be 400–550 (Reijnders *et al.* 1993). Historical records suggest that Mediterrranean monk seals used to rest and breed on open sandy beaches and rocky shelves, but now this species is only seen on beaches within caves on isolated and inaccessible coasts. It still occurs throughout its historical geographic range but its distribution is highly fragmented and surviving populations are separated by large areas of probably unsuitable habitat. In the early 1950s this species occurred from Cap Blanc in Mauritania to the Black Sea, with small populations on the Spanish and French mainlands, in Corsica, and an almost continuous distribution along the north African coast in the western Mediterranean. By the 1970s monk seals had disappeared from the French coast and much of the Spanish and Algerian coasts. The most severe fragmentation has occurred during the last two decades when this species has disappeared from 10 countries. As predicted by Durant & Harwood (1992), the Black Sea and western Mediterranean populations are now virtually extinct. The only viable

populations are in the Atlantic and the eastern Mediterranean, and these are separated by 4500 km. The species now has a non-equilibrium metapopulation structure, with local extinctions plus an overall decline in numbers (Harrison 1991; Harwood *et al.* in press).

Threats to populations

Although deliberate killing (largely by fishermen) and loss of suitable habitat for pupping are the primary reasons for population fragmentation in most of Europe, these factors are not currently important on the Atlantic coast, where all breeding is now concentrated along less than 20 km of coastline on the border between Mauritania and Morocco and on a small group of islands in Madeira. These colonies are particularly vulnerable to the effects of infectious disease, oil spills, and cave collapse. The Atlantic monk seal population probably experienced a genetic bottleneck in the 18th century (Monod, in Marchessaux 1989) lasting at least 10 generations. Founder effects and genetic drift may have created genetic differences between seals here and in the Mediterranean.

In the eastern Mediterranean monk seals are widely dispersed in a large number of local populations each containing 10–40 individuals. This region is a popular tourist destination and disturbance levels are high. However, deliberate killing by fishermen has recently been identified as the greatest threat to monk seal survival in this region (IUCN & Species Survival Commission 1994).

Conservation measures

In-situ protection, through the establishment of protected areas which incorporate known pupping sites, is the preferred approach to help save the species from extinction. However, to date only two protected areas have been established: the Desertas Islands, Madeira; and the Northern Sporades, Greece. Within these areas seals are legally protected but enforcement of protection can be difficult. Under these circumstances, captive breeding, with a view to future re-introduction, must be considered as a conservation option. The European Commission has supported a study of the feasibility of captive breeding for *M. monachus*, thereby implementing an IUCN recommendation that if less than 1000 animals remain in the wild, a captive breeding programme should begin (Reijnders *et al.* 1993). However, this project has now been abandoned because of opposition from some parts of the scientific community.

The seal population on the Moroccan/Mauritanian coast is a potentially important source of immigrants to re-establish the population in the Canary Islands and also to reinforce the population in Madeira. Active translocation of seals to the Canary Islands is now being considered as a way of reducing the overall vulnerability of the species in the Atlantic and to improve gene flow between the African coast and Madeira. However, there is a clear need to identify the potential risks to the African population from translocation, and to determine whether improved gene flow is actually necessary, before implementing such conservation action.

A rescue network and rehabilitation centres for abandoned pups have been established in Greece, and healthy seals have been released back into the wild.

Role of genetics

Conservation initiatives, particularly those involving captive breeding, translocation or reintroduction, require an understanding of the ecology, behaviour and genetics of the species. Species-specific data on the extent and distribution of genetic variation are important to help minimise problems caused either by inbreeding or outbreeding. We investigated the level

of genetic variability within and between monk seal populations using mitochondrial DNA (mtDNA) sequence analysis. mtDNA has a high rate of evolution and is maternally inherited and, due to their haploid nature, genotypes can be lost or fixed relatively rapidly. The potential for finding mtDNA population markers is therefore high and this approach has been widely used to determine relationships among closely related populations (e.g. Avise 1994, 1995; Moritz 1994).

Methods

Samples

Samples of skin, hairs and cultured lymphocytes were available from the north Atlantic (n = 10–33 – some samples were of hair or rotting skin and so it is not possible to know exactly how many individuals are represented), eastern Mediterranean (n = 19), and western Mediterranean (n = 1).

DNA extraction and analysis

Total genomic DNA was extracted using standard procedures (Sambrook, Fritsch & Maniatis 1989) or with the addition of a high concentration of DTT (Paabo 1989). We amplified samples using the polymerase chain reaction (Mullis & Faloona 1987; Saiki *et al.* 1988) and sequenced 350–444 bp of the control region (CR) from 38 samples and 350 bp of the cytochrome b (cyt b) gene in selected individuals. We also investigated the evolutionary divergence between *M. monachus* and *M. schauinslandi* by sequence analysis of the complete cytochrome b gene. Sequences were aligned using the program Clustal V (Higgins & Sharp 1989).

Results

Control region

Only two variable sites (transitions) were identified which defined two genotypes (genotypes 1 and 2). Sequence divergence between genotypes was less than 1%. Genotype 1 was found in the one animal from the western Mediterranean and in all north Atlantic samples. All samples from the eastern Mediterranean had genotype 2. The CR from *M. schauinslandi* was approximately 17% divergent from *M. monachus*.

Cytochrome b

No intraspecific sequence variation was detected in 350 bp of cyt b. Sequence comparisons using the full gene showed that divergence between *M. monachus* and *M. schauinslandi* (12.8 %) was almost as high as the divergence found between phocid genera.

Discussion: the implications of genetic data

Small, but possibly fixed, differences in CR genotype exist between the two remaining populations of *M. monachus*. These data suggest that there has been limited gene flow between these two monk seal populations but do not support designation of a distinct subspecies in the Atlantic. The low level of CR sequence divergence (less than 1%) is in line with data obtained from other marine mammals, particularly the northern elephant seal *Mirounga angustirostris* (another monachine) which is known to have experienced a genetic bottleneck in recent history (Hoelzel *et al.* 1993; Hoelzel, Hancock & Dover 1993). Mitochondrial genotypes can be markedly affected by fluctuations in population size and habitat fragmentation over relatively short periods. Data should therefore be interpreted with caution when knowledge of population histories is limited.

Although we require additional samples from the Atlantic, our results suggest that seals in Madeira and the African coast have very similar mitochondrial genotypes and are therefore likely to be genetically compatible. As is the case in

other mammalian species with minimal CR sequence divergence, no variation within or among populations was detected in the cyt b gene. Our data therefore suggest that the two major monk seal populations are also genetically compatible. However, so long as seal numbers in the Atlantic and eastern Mediterranean do not decline dramatically it may be preferable to maintain the genetic integrity of the two populations in case there are undetected adaptations to local conditions in these populations.

Interspecific comparisons using the cytochrome b gene show a large divergence between *M. monachus* and *M. schauinslandi*, which is in agreement with what we know of their evolutionary history. This implies that care should be taken in using data from *M. schauinslandi* to develop conservation programmes for the highly endangered but poorly studied *M. monachus*.

The observation that there are single mtDNA genotypes in the Atlantic and in the eastern Mediterranean population suggests that there is very low intrapopulation genetic diversity. However, mtDNA provides only limited information on intrapopulation variation and it is desirable that the identification of population groupings is supported by independent data sets. Thus, the analysis of nuclear variation (both within and between populations) is an important next step in this study. The information obtained from limited numbers of samples can be increased by analysing many microsatellite loci. A study of genetic variability at nuclear loci using microsatellites will help us to establish whether the mtDNA result is a true reflection of the overall level of genetic variability in *M. monachus*.

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Wildcats: can genetics help their conservation?

D. Balharry, M. Daniels and E.M. Barratt

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The identity of the wildcat has been contentious over time, leading to concerns about its conservation status. The taxonomic basis for conserving the wildcat presents a dilemma in that 'species uniqueness' may not exist in an animal which has hybridised with its domesticated form. An alternative may be to define the wildcat by linking it to its habitat or defining what is not a domestic cat.

Recent morphometric research has shown that it is possible to identify two groups of cats. However, within these groups there is a large degree of overlap in key variables. The groups could not be separated on the basis of pelage markings alone. Genetic variability between the groups was measured for nine nuclear microsatellites. All nine loci contained private alleles for one group or the other but these were of a low frequency and not agglomerated, in contrast with other studies on closely related interbreeding species. Variation between groups was generally low but significant for some loci. The differences could not be explained by geographic partitioning and it was concluded that there was a very limited degree of genetic distinction between the groups.

The evidence presented suggests that there may be little justification for considering wildcats and domestic cats as separate species. Consequently, the use of the terms 'hybrid' and 'pure' are unhelpful. The implications of both morphological and genetic research are that a discrete definition for the wildcat may not be possible.

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Introduction

The identity of the wildcat has been a contentious issue for over a hundred years (Darwin 1868; Hamilton 1897; French *et al.* 1988; Easterbee, Hepburn & Jeffries 1991; Balharry & Daniels in press). The confusion hinges on the wildcat's reproductive relationship with feral and domestic cats. Recently, concerns regarding the conservation status of wildcats throughout Europe have heightened interest in the debate (Anon. 1992). Formal taxonomy classifies wildcats as a different 'species' from domestic cats, yet interbreeding between the two species has long been recognised (Anon. 1771; Darwin 1868). Taxonomic status is used as the starting point for assessing the conservation value of most species. However, in the case of the wildcat, where it is likely that the species has been misclassified, a different approach to assessing its conservation value may be required.

This paper reviews the background issues to the debate, provides an early indication of the results of recent and ongoing research, and considers the implications for the future conservation status of the wildcat. The paper is written in four parts. The first part looks at the changing identity of the

wildcat in an attempt to expose the reasons for the current confusion and the impact this has on the legislative position. The second part considers the difficulties associated with using a taxonomic approach to assessing conservation value for species which have domesticated forms. The third part reviews the results of recent morphometric and genetic research. The final part discusses the contribution of the genetic research to the debate, and possible implications for the conservation of the wildcat.

The changing identity of the wildcat

Ancestral period

The 'Ancestral' is the earliest definition of the wildcat and it spans *c.* 8000 years in Britain without contention. About 10,000 years ago the land bridge connecting Britain to Europe was cut by rising sea levels and the wildcat *Felis silvestris*, along with the lynx *Lynx lynx*, were the two felids left behind (Yalden 1982). The population is thought to have stretched from northern Europe (including Britain) to South Africa.

The domesticated cat probably originated about 5000 years ago in Egypt, either through selective breeding or by a passive process of adjustment to living alongside human communities (Todd 1978). Whatever the mechanism, 'domestic cats' came to be characterised by an increase in the frequency of multiple coat colours and patterns alongside the maintenance of juvenile behaviour into adult life, a trait that is commonly associated with social tolerance (Hemmer 1990). In the 'normal' evolutionary environment (i.e. devoid of man) it is unlikely that these characteristics would have imparted a selective advantage and indeed it could be argued that they had a positive disadvantage. However, their persistence in domestic and feral cats today implies that the traits may impart a selective advantage for cats living in association with man.

The domestic cat arrived in Britain *c.* 2000 years ago with the spread of the Roman Empire (Serpell 1988; Clutton-Brock 1989). Its presence created the possibility of interbreeding with wildcats and from this point on the ancestral definition was undermined.

Historical period

For this period, which we have defined as 2000 BP to 100 BP, the definition of a wildcat is associated with its name, rather than a description. A recent literature review noted an increase from the 18th century in the occurrence of the prefix "wild" and the increasing use of the descriptive terms "ruggedness" and "fierceness" by authors referring to what they believed to be the (by then) only native felid (Lamb 1995). This information lends support to the view that domestic cats were an established part of Highland life at the time of the clearances (1790s–1830s).

During this period the characteristics of both the ancestral cat and the feral cat (domestic cats living wild) may have been evolving in response to a changing environment. Studies of domestic cats introduced onto islands have shown that they are highly adaptable and capable of surviving in a wide variety of environments, for example Australia, New Zealand and sub-Antarctic islands (Newsome 1991; Derenne 1976; Derenne & Mouglin 1976; van Aarde & Blumenberg 1979; Fitzgerald 1988). It has been noted that in these circumstances the frequency of the 'domestic' characteristics in the population is reduced. The evolutionary environment of the ancestral wildcat was also changing due to differing land use by an expanding human population, especially through widespread deforestation and increasing cultivation. During this period one of the most significant changes was the addition of rabbits to the prey base. Although introduced to Britain in the 12th century, rabbits only colonised the north of Scotland during the 19th century

(Thompson 1994). The precise effect of the rabbit introduction on evolutionary selection pressures is unknown but given the importance of rabbit in the diet of wild-living cats today, this factor cannot be overlooked.

In summary, during this period it is reasonable to assume that the characteristics of both the feral and the ancestral cat were evolving to cope with a new set of environmental conditions. Importantly, the new conditions were the same for both 'populations' and there was also the potential for introgression of genes between both 'populations'.

Traditional period

The potential detrimental effect of interbreeding between wildcats and domestic cats for the native population was acknowledged by Hamilton (1897) who, after thirty years of study, wrote:

"*The history of the Wild Cat of Scotland must be in a great measure conjectural. . . . I find but very few facts which can be entirely relied on and the few facts which can are mixed up with a great many fallacies. It would seem that the original Wild Cat, . . . has for a long time been quite extinct in this country, its place being taken in the first instance by a mixed breed, . . . in which the foreign characteristics of the ancestral progenitors of the domestic race, viz. the African cat, were in the ascendant and prevail up to the present time.*"

Seven years after Hamilton expressed concerns regarding the identity of the wildcat an animal from Invermoriston, Highland, was classified as the 'type specimen' for the Scottish wildcat (Pocock 1951). The source for much of today's controversy stems from the way in which this 'type specimen' was used as a bench-mark against which future samples were collected (Dadd 1970). Not only was the possibility of 2000 years of interbreeding between wild and domestic cats overlooked, but also the description was arguably based on too few samples. The problem was further compounded when the museum purchased cats to enhance the collection. Those that failed to conform to a tight description were rejected or labelled 'hybrids', despite the knowledge that they had originated from the same geographical area.

As a result, today the Natural History Museum has a large collection of 'wildcats' with remarkably similar markings! The 'traditional definition' of wildcats is based on this collection. The 'traditional definition', with the further addition of an observer credibility factor (credibility of reports depending on the occupation and location of the recorder) was the basis for determining the status of wildcats (Taylor 1946; Jenkins 1962; Easterbee 1991). Reports on the changing status of the wildcat formed the basis for its protection in 1988. However, the only attempt to enforce the legislation on the basis of the traditional definition, in the Stonehaven Sheriff Court in 1990, was unsuccessful.

Current legislative position

Internationally, the 'wildcat' is perceived as a species under threat and in decline throughout its range (Stahl & Artois 1994) and hence is subject to strict national and international protection measures (Annex IV of the EC Habitats Directive, Appendix II of the Bern Convention, Appendix II of CITES, and Schedule 5 of the Wildlife and Countryside Act 1981). Ongoing confusion with regard to the difference between wildcats and feral cats has allowed deliberate persecution to continue throughout the range despite the legal protection. The ineffectiveness of existing legislation is internationally recognised; a Bern Convention European seminar on wildcats (Anon. 1992) identified three main reasons for the decline of the wildcat, namely:

hybridisation of the 'wildcat' with feral or stray cats;

difficulty in distinguishing, in some circumstances, wild from domestic cats or those from hybrids;
frequent shootings of 'wildcats' in areas where the shooting of feral cats is permitted.

In view of these findings conservationists throughout the former range of the ancestral wildcat debated what could be done to conserve the species (Stahl & Artois 1994). During these discussions considerable hope was expressed that genetical techniques could be used to identify which populations are closest to the ancestral type.

Conservation

In order to progress the debate on the identity of the wildcat and its conservation status, it is worth reflecting on what it is that we wish to protect. Few argue with the statement that conservation is a relatively subjective science. Generally, in the context of species, the measures used to guide the subjective debate are either uniqueness or the contribution of the individual or population to the ecosystem. For the purpose of informing the debate on wildcats some background issues need to be explored before attempting to define uniqueness and conservation value in 'wildcat' terms.

Broadly speaking the scientific approach to assessing the uniqueness of a species for conservation involves the use of one or more of three criteria: morphometrics (size, shape, colour, etc.), behaviour (especially vocal communication) and, more recently, genetics. When the identity of an animal has been agreed, its population can be censused, its relative rarity determined and conservation importance agreed. This approach is practical and works well for most species. However, it has two limitations when applied to species which hybridise or have domesticated forms. First, despite the objective intention, it does expose the tendency of the human mind to simplify diversity into *discrete* entities. Francis Bacon in his *Novum Organum* (published in 1620) described this as a natural tendency to generalise on the basis of all too slender information and find order where there is none.

Most species conservation progresses on this basis, despite the problems associated with applying practical conservation measures to basic theoretical frameworks (see Daugherty *et al.* 1990). In an attempt to force the classification of some animals into discrete entities, higher resolution techniques are continually being developed, e.g. the analysis of the echo-location pattern of pipistrelles suggests that there may be two different groups living sympatrically; skull morphology, which is traditionally measured using calipers, is now being assessed using scanners; species translocations are preceded by genetic screening. Given this search for an objectively defined discrete entity, it is likely that the debate on the conservation management of these species will be driven by financial constraints or practical needs rather than by scientific enquiry.

Alternative approaches to discrete entities have been advanced. For example, a recent review by Balharry *et al.* (1994) suggested that the conservation value of some species would be better defined in terms of the habitats that they occupy. The logic of this argument is simple: if species are inextricably linked to their habitats (captivity excluded), and it is accepted that habitats and selection pressures are not static, then we would expect the characteristic of species to change with time (i.e. the discrete entities of today may be inappropriate in the future), especially if the species concerned is both polymorphic and a generalist (Balharry *et al.* 1994). The public might then logically conclude that the species "is not as pure as it used to be" or that "it no longer exists". Such conclusions overlook the fact that the animals are still living and breeding in their habitat.

The wildcat, however, has a strong cultural and historical image beyond that of the framework used by scientists. Its conservation value to many is as an emblem of the Scottish Highlands, used in the crest of Clan Chattan, and the subject of many traditional Highland tales (St John 1849). It is unlikely that in the short term new scientific research will change these long-established values.

In this context it is possible to pose three alternatives for assessing the conservation value of the wildcat. These are:

- a definition based on discrete and recognisable groups;
- a definition linking the animal to its habitat;
- a definition based on what the domestic cat is not.

Current and ongoing research

Morphometrics (1993–1995)

Previous research designed to refine the definition of 'wildcats' started with the premise that the researchers knew beforehand which cats were wildcats, i.e. they relied on the traditional definition. The majority of this research sought to define 'hybrids', but inadvertently statements about the identity of the wildcat were made in an attempt to bolster the cracks in the traditional definition, e.g. wildcats are heavier, larger and have relatively shorter tails (Kitchener & Easterbee 1992; Corbett 1979; Schauenberg 1977). Skulls of cats came in for particular attention with every possible knob, process and bump (skull morphometrics) being measured in an attempt to differentiate between domestic, wild and hybrid forms (Ragni & Randi 1985; French, Corbett & Easterbee 1988; Fernandez, de Lope & de la Cruz 1992). On the basis of skull morphometrics French, Corbett & Easterbee (1988) developed a key that classified cats into three groups that they subsequently labelled domestic, wild and hybrid.

In an attempt to avoid the difficulties experienced by previous researchers, the current research set out to test the hypothesis that there is only one population of wild-living cats in Scotland, i.e. that there is no substantive difference between wild and feral cats. The aim was objectively to measure and assess key variables. Over 200 cat carcasses were collected. Limb bones were measured to the nearest 1 mm; epiphysial ossification of limb bones and dentine rings were used to assess relative age; the length of the intestine was measured to the nearest 1 cm; skulls were assessed using the key developed by French, Corbett & Easterbee (1988); and pelage characteristics were measured on the basis of 27 variables: body colour and pattern (10 variables), tail shape and rings (7), leg and feet markings (6) and body stripes (4). The objective assessment of these data was then compared with the subjective assessment of an 'expert' who classified the cats broadly along the lines of the traditional criteria.

The results demonstrated that pelage markings could not be used in isolation to create biologically meaningful groups. The skull measurements gave no information other than overall size. Comparison with the key of French, Corbett & Easterbee (1988) revealed only 57% agreement with the 'expert' assessment. The only data to suggest some degree of bimodality were the measurements of intestine length and bone size. Plotted against each other they revealed a significant negative correlation (Figure 1). On this basis it was possible to divide the sample into two statistical groups (Balharry & Daniels in press). Group 1 consisted of large cats with small intestines and a preponderance of pelage markings traditionally associated with wildcats. Group 2 was characterised by smaller cats with long intestines and more frequent pelage markings associated with domestic cats. Simplistically the characteristics of group 1 were closer to the traditional description of a wildcat

and group 2 cats are closer to 'domestic cats'. However, in reality the relationship between group 1 and group 2 was a complex cline of overlapping variables. Analysis of the geographical distribution of the groups revealed that group 1 cats were more likely to be found in higher, colder and (relative to west coast areas of similar altitude) drier areas with poorer capacity for forestry and agriculture, suggesting that the morphological variability detected in the analysis may be the result of present day environmental selection pressures (Balharry & Daniels in press).

Two hypotheses can be advanced to explain the two groups and their large overlap in the key variables. First, there may be a single population with a large phenotypic variation sustained by different selection pressures acting in different environments. Second, there could be two populations with considerable overlap of some characteristics. There is no information to link either group to the ancestral type. Taking into account the environmental changes that have taken place in Scotland over the last 2000 years (including the arrival of domestic cats and subsequent interbreeding, the arrival of rabbits and climatic change), the groupings may be more the result of current day selection pressure rather than the discovery of a refugium of ancestral-type cats.

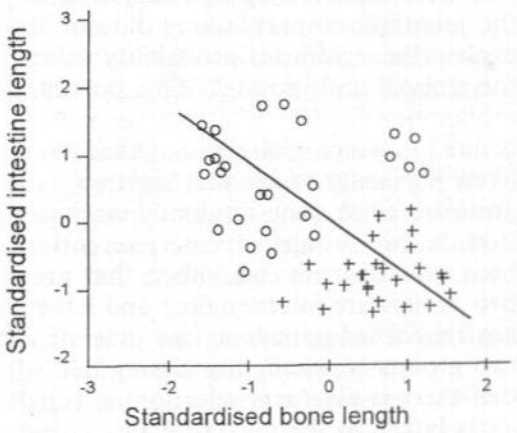

Figure 1. Bone size and intestine length in 52 adult cats. To take account of differences between males and females, bone and intestine measurements were standardised by subtracting the mean for the appropriate sex, then standardising the residuals so that they had unit standard deviation. There is a correlation coefficient of –0.30 ($P < 0.05$). The "o" represents animals classified as group 2 and the "+" animals classified as group 1.

Morphometrics as a method for identifying discrete populations has been severely criticised as a taxonomic tool, because of problems associated with phenotypic plasticity (Geist 1992). It therefore remains important to advance, alongside the morphometric studies, a genetic and ecological understanding in order to clarify the significance of the groups. The ecological relationship between the two groups in the wild is the basis of an ongoing study.

Will genetics help?

There has been a tendency recently for taxonomists, ecologists, conservationists and politicians to view genetics as the panacea that will answer all problems (Avise & Hamrick 1995). However, genetic research is merely a tool with which to test a hypothesis and not a solution in itself.

Essentially, for wildcats the genetic question is based on the same theme: is there a genetic difference between two predefined groups, between different geographical areas, or between cats classified into groups by 'experts'? If genetically distinct groups are found then the possibility of testing to determine the closest descendant of archaeological or historical samples remains. The genetic variation within groups 1 and group 2 (Figure 1) has been analysed.

Genetic variability between the predefined groups was measured using 9 specific nuclear markers (microsatellites). These are short lengths of DNA which can be amplified easily using PCR (polymerase chain reaction). This has

Table 1. *Fst* values and attendant probabilities for the differences in allele frequency between groups 1 and 2. Probabilities (P) less than 0.05 are deemed to be significant.

	Microsatellite loci								
	Fca 8	Fca 23	Fca 35	Fca 43	Fca 45	Fca 77	Fca 90	Fca 96	Fca 126
Fst	0.01	0.01	0.02	−0.01	0.02	0.02	0.02	0.004	0.06
P	0.06	0.04	0.04	0.28	0.03	0.11	0.2	0.2	0.001

distinct advantages in that DNA from degraded samples, such as those found in hair and bone tissue of cats in museums, can be analysed. All samples were screened using predefined microsatellites (Menotti-Ramond & O'Brien 1995). Analysis of the data provided information on the genetic variation between populations (*Fst* values, Wright 1969) and within population (*Fis* values).

Microsatellite analysis has been used to provide data on the direction and extent of hybridisation in a number of studies. In the case of the red wolf, microsatellite markers developed in dogs were used to screen population samples from red wolves, grey wolves and coyotes. The red wolf individuals had microsatellite alleles found in both coyotes and grey wolves; no unique alleles were found. This led Wayne & Jenks (1991) to the suggestion that the red wolf is a hybrid of the coyote and grey wolf. In the case of the simian jackal, hybrid individuals were identified by the presence of unique domestic dog alleles, which were absent from pure simian jackals, or Ethiopian wolves as they are now known (Gotelli *et al.* 1994).

In the cat study DNA was extracted from over 200 samples and 9 polymorphic microsatellite loci were amplified and scored using radioactive markers. All 9 loci contained private alleles (present in only one group, population or species) for either group 1 or group 2. However, they were generally of low frequency and there was no agglomeration of alleles in either group, as observed in the microsatellite data from the red wolf study.

The variation in the allele frequencies between the two groups was analysed, and *Fst* values estimated for each locus. *Fst* values were all generally low; four out of the nine were significant (Table 1). The biological inference from such *Fst* values is, however, difficult to establish. Although some of the differences are significant, the absolute *Fst* values are lower than, for example, those found between human populations in Scotland and Wales.

To determine if these *Fst* values were due to chance groupings in a substructured population, the samples were re-divided into geographical groups based on the Regions of Scottish Natural Heritage and *Fst* values were recalculated. The results from this exercise are interesting and suggest that the geographical partitioning did not explain the significant probability values for group 1 and group 2. One possible explanation for the differences observed is due to chance groupings. Other less likely explanations are that the two groups are not quite randomly mating; that the two groups have very recently been isolated from each other; that the two groups are intermingling and have nearly reached panmixia; and that the two groups are randomly mating but that there is divergent selection at a locus linked to the microsatellite.

This analysis is at a relatively early stage and, as such, the addition of further samples, including museum specimens, may alter the explanations. Additionally, alternative methods for structuring the expanded data set may yield radically different results. However, on the basis of the present study, the small but

significant *Fst* values suggest a very limited level of genetic distinction between the two groups.

Discussion

There is a dilemma for the conservation of the wildcat, which is a 'species' with a large range (Scotland to southern Africa and western Europe to western China) across which there is considerable clinal variation. Throughout most of this range the 'domestic form' lives in sympatry. In many parts of its range the feral/domestic cat significantly outnumbers wildcats. In addition feral/domestic cats can be, and are, legally killed. Given that there is no known way visually to distinguish between them, wildcats may be persecuted and this causes concern with regard to their current conservation status. Also the effects of ongoing introgressions with feral/domestic cats are unknown. Essentially, if the animal cannot be clearly defined, can its conservation be secured?

The analysis of the phenotypic variability of the sample indicated two groups of cats, between which the difference was statistically significant. These differences were determined using multivariate analysis on a combination of different variables. The groupings were also found to correlate with environmental variables. However, in both groups there was a mix of different coat colours and patterns, for example black cats and tabby cats with white.

Analysis of the genetic variation between the phenotypic groups revealed significant differences. However, while statistically significant, these findings may be considered in the light of other analogous genetic studies. For example, the level of variation reported here is below that found between populations within other species and between many closely related species. The evidence implies that there may be little genetic justification for considering wildcats and feral cats as separate species on the basis of the phenotypic groups 1 and 2. This being the case, the use of terms such as 'hybrids' and 'pure' can be regarded as unhelpful in the context of describing the identity of the wildcat in relation to domestic cats. Genetically, the sample may be one population in which the phenotypic expression of the genes appears to be strongly determined by some environmental conditions.

The implications of the recent research are that a discrete definition for wildcats may not be possible. Therefore the first proposal for assessing the conservation value of wildcats, 'a definition based on discrete and recognisable groups', may be discarded. What is it that we value and what is it that the legislation is attempting to protect?

Two alternatives to the discrete groups were proposed at the start: a definition *linking the animal to its habitat* and a definition based on *what is not a domestic cat*. The results of the research indicate phenotypic and genotypic variation correlated with environmental conditions. The implication is that the variance may be due to different selection pressures acting on the population in different areas. The final decision regarding the identity of the wildcat must now be taken forward by scientists working closely with policy makers and politicians.

There do, however, remain some conundrums; the policy and political debate hinges on the 'threat' and what legislation can do to minimise its effect. At a population level, the cats living wild in Scotland are phenotypically different from domestic cats (as would be expected given the different selection pressures acting on the populations). A wide range of histories surrounds individual cats within the wild-living population, e.g. some until recently may have been domestic, others may be descendants of domestic cats several generations back and other may be descendants of wildcats and yet others may be any combination of the above.

The mix in any geographic area is likely to depend primarily on two important selection pressures, environmental and population control/persecution by humans. For the latter, 'selection pressure' may be a slight misnomer since feral cat control is both unselective and effective, with the likely consequence being that recruitment from adjacent populations will increase. If these adjacent populations have a high proportion of domestic traits then their introgression into the wild population may have a negative effect on the ability of the animals to survive in the harsher environment. Given that cessation of shooting feral cats across the country is unlikely to be acceptable to estate managers, any legislative changes will have to be carefully considered; indeed there may be a need to consider management of domestic cats in a way that minimises interbreeding with the wild population.

Scientists will need to contemplate two further conundrums. How can the long-term effects of continued introgression of domestic traits be assessed? How can 'favourable conservation status' be monitored? Without agreement on the identity of the wildcat, neither of these questions can be answered.

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Genetic aspects of the Species Recovery Programme for the Plymouth pear *Pyrus cordata* Desv.

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Jackson, A., Erry, B., & Culham, A. 1997. Genetic aspects of the Species Recovery Programme for the Plymouth pear (*Pyrus cordata* Desv.). *In*: *The role of genetics in conserving small populations*, ed. by T.E. Tew, T.J. Crawford, J.W. Spencer, D.P. Stevens, M.B. Usher & J. Warren, 112–121. Peterborough, JNCC.

Genetic factors have been pivotal to the Species Recovery Programme for *Pyrus cordata*. The primary target was to safeguard the two existing populations by ensuring long-term viability. Using the molecular technique of Randomly Amplified Polymorphic DNA (RAPD) only two clones have been identified, one from each of the two reproductively isolated populations in south-west Britain. This low level of genetic diversity was illustrated by the results of controlled pollinations within and between these populations. Self- and cross-incompatibility was exhibited within each of the populations, where individuals were separated by up to 1.9 km. However, when crossed, the two reproductively isolated populations were compatible. With only two clones, minimum allele frequencies at any locus in the British populations are 0.25. This means that a maximum of four or a minimum of three *S* alleles are controlling self-incompatibility. The loss of a single *S* allele could have a significant effect upon future reproductive performance and therefore the medium-term conservation of the British representatives of *P. cordata*. To conserve the remaining genetic diversity and thereby ensure long-term viability, an experimental population was designed to bring together the two genetically distinct and currently reproductively isolated clones. The first phase of planting commenced in 1995. In the longer-term, it is intended to review this strategy following a study of the genetic diversity of *P. cordata* throughout its distribution in Europe.

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Introduction

The Plymouth pear *Pyrus cordata* Desv. is one of Britain's rarest wild trees. It is the only tree species to be protected under Schedule 8 of the Wildlife and Countryside Act 1981. In 1991 it became one of the first six taxa in the Species Recovery Programme managed and funded by English Nature. Its selection was justified on the basis of its rarity in Britain, its statutory protection and concern for the security of the wild populations in the long-term. As a wild relative of the cultivated pear *P. communis*, with which it can freely hybridise, it is also worthy of conservation interest as a potential genetic resource for future breeding programmes.

The overall strategy of the recovery programme for *P. cordata* combines *in-situ* and *ex-situ* initiatives which are founded on an understanding of the complex, interacting disciplines of genetics, reproductive biology, ecology and horticulture (Jackson 1995). Genetic factors have been pivotal to the recovery programme and their detailed consideration was essential for the design of a medium-term conservation strategy.

The distribution of *Pyrus cordata*

Pyrus cordata is naturally restricted to south-western Europe with populations in France, particularly Brittany, and the north-west of Spain and Portugal. The two populations known in south-west Britain are its most northerly locations and, although geographically disjunct, are linked to mainland populations by similarities in climate, flora, geology and history of human disturbance. Human occupation of this region has resulted in a predominantly agricultural landscape interspersed with larger blocks of semi-natural and planted woodland which are interconnected by hedgerows and riverine woodland.

The British populations

Anthropogenic habitats dominate the British populations. In Plymouth, Devon, the once rural hedgebank where it was first discovered in 1865 (Archer Briggs 1880) is now subsumed within a light-industrial estate. Despite local protestations, a significant proportion of the original hedgebank was destroyed during development in the 1970s. In 1989 a second hedgebank was found 1.6 km to the east and today a total of 20 individual stems have been uniquely numbered from within these hedgebanks and the several individuals which were transplanted in the 1970s. Numerous small suckers occur within the dense hedgerows and 28 of these have been transplanted to semi-natural areas managed as Local Nature Reserves by Plymouth City Council.

In Cornwall five sites have been located near Truro, separated by up to 1.9 km. The first of these was confirmed from voucher specimens collected in 1989 and the last was found as a result of publicity in the local press in 1991. Over 80 individual stems ranging from 1 cm to 46 cm diameter have been individually tagged and 450 other small plants have been recorded and mapped (Tonkin 1993).

Owing to the ability of *P. cordata* to produce an abundance of suckers it is often difficult in the field to identify what should be regarded as an individual plant worthy of a unique number. Furthermore, segregating the population into sexual and asexual propagules is totally impractical. Attributing an age to individuals, as estimated by stem diameter and height, must also be considered in the light of this asexual reproduction and it is likely that the individuals are much older than their measurements might indicate because of the impact of management events such as hedge-cutting.

Threats to the British populations

All but one of the British sites of *P. cordata* are protected within Sites of Special Scientific Interest (SSSIs). However, unintentional damage can still occur through agricultural practices such as hedge-cutting, grazing or pesticide drift. The provisions of the Wildlife and Countryside Act should reduce the likelihood of the wholesale habitat destruction that occurred to the Plymouth population in the 1970s. The theft of the entire fruit production from controlled cross-pollinations in 1994, however, is an indication of one of the more subtle human pressures. Pathogens such as fireblight *Erwinia amylovora* have the potential to eradicate entire populations quickly and must be constantly monitored.

Most of these factors are of short-term importance. If *P. cordata* is to become self-sustaining in the long-term, then the greatest concern must centre around its ability to adapt to changes in the local environment. This will be dependent largely on the genetic variation of individuals within populations arising through propagation by seed. Therefore, reproductive isolation, leading to low seed-set, and inbreeding depression where incompatibility occasionally breaks down, are both immediate and long-term threats to the genetic heterogeneity of the populations.

The need to identify genetic variation

Considerable morphological difference was identified between the Plymouth and Truro populations. This included leaf shape, flowering time, carpel number and the overall form of the trees. On morphological evidence alone the two populations were thought to be genetically distinct. Indeed, initial concern focused on whether both populations were the same taxon. This was resolved only after a thorough literature review and on comparison with herbarium specimens from mainland Europe. Variation within each population was restricted to flowering time and growth habit. Much of this was apportioned to differences in the local microclimatic and edaphic conditions.

An early indication of a low level of genetic variation within the British populations was gained from the results of both open and controlled pollinations. Before the Recovery Programme began the annual fruit production of the Plymouth population had been observed to be sporadic. Records of viable seeds existed but no detailed accounts were available. Then, in 1992, an unusually large harvest of 1178 open-pollinated fruits yielded only 19 fully developed seeds. Germination tests on Plymouth seeds indicate 80% viability (Jackson 1993), but the seedlings are not vigorous. A study of pollen tube growth confirmed that self-incompatibility, by prevention of normal pollen tube development, prevented fertilisation within the Plymouth population. Controlled pollinations confirmed this incompatibility with 172 self-pollinations and 141 pollinations between individuals producing no viable seeds.

This result indicated the need to employ a DNA fingerprinting technique to identify genetically distinct individuals in order to direct a hybridisation programme.

It was necessary to study genetic diversity at three primary levels:

- among individual plants;
- among sites within the populations;
- between the two main populations (Truro and Plymouth).

In addition, hybridisation experiments demonstrated that viable seed could be readily obtained by crossing *P. cordata* with a wide taxonomic range of wild pears and with cultivated varieties of *P. communis* such as 'Williams' and 'Conference'. Given that some seed had been obtained through open-pollination in the Plymouth population there was further concern about the possibility of introgression and loss of distinctiveness between the wild populations of *P. cordata* and cultivated pears nearby. The final question was, therefore, whether true *P. cordata* could be identified at the species level using genetic markers.

DNA fingerprinting

The complex distribution of the British populations and the difficulty of identifying unique individuals in the dense hedgerows presented practical problems in deciding which plants to sample and how to identify them. These decisions were essential precursors to the DNA analysis given that the aim was to return to the plants to conduct a cross-pollination programme. Over 120 of the largest individual stems were uniquely numbered and more than 460 other small plants were recorded and mapped. The 120 numbered plants were used for sampling. Fresh leaves were gathered from each and duplicate DNA extractions made for each numbered individual.

There are several plant genetic fingerprinting techniques available, each requiring a slightly different knowledge of the organism under study and each with particular limitations in terms of time, cost and quantity of DNA required. The smallest plants had only ten leaves and availability of DNA was

therefore one of the primary limiting factors.

One of the most widely used fingerprinting techniques, Randomly Amplified Polymorphic DNA (RAPD) (Welsh & McClelland 1990; Williams *et al.* 1990) was considered particularly applicable to this project for the following reasons.

- The quantity of leaf material, and therefore quantity of DNA available, was very small for many individuals.
- Sampling needed to be as non-destructive as possible. With certain techniques this requirement would have limited the sample size so severely that the between-individual genetic variation could not have been investigated.
- The number of individuals to be screened was large, and both time and resources were limited.
- The plants under study are long-lived woody perennials and particular banding patterns could, therefore, be associated with particular plants for the full duration of this project, and throughout the ensuing Recovery Programme.

The use of the M13 probe (Ryskov *et al.* 1988) for DNA fingerprinting was evaluated, but too many bands were generated for easy comparison of individuals. Also, the relatively large amount of DNA required per individual for one trial (11 µg) was far more than could be extracted from small plants if the patterns were to be confirmed by repetition of the experiment.

RAPDs require little DNA because the procedure amplifies the target DNA; it requires no prior knowledge of the genome because the primers are random; and the technique can be applied to large sample sizes very quickly (see Berry, Crawford & Hewitt (1992) for an overview of techniques and applications). The resolution of the technique is somewhat governed by the number of primers that can be screened and, in most cases, banding patterns show dominance so heterozygotes cannot be distinguished. The variation in banding patterns is therefore largely that of presence or absence rather than band shifting.

Three sets of primers of different origin were used: Williams *et al.* (1990) – 10 primers; Operon Technologies Inc. (Set E) – 20 primers; University of British Columbia (UBC) (Set 8) – 100 primers. This final set was chosen as a result of reports indicating that they were more sensitive at detecting genetic polymorphism.

Samples were taken from 123 numbered plants ranging from mature trees to stems less than 20 cm tall. DNA was extracted from either fresh leaves or leaves dried by silica gel, using the CTAB extraction method (Doyle & Doyle 1987). DNA quality and quantity were assessed prior to dilution suitable for the polymerase chain reaction (PCR).

The first 75 DNA extractions, representing both of the main Plymouth sites and all five Truro sites, were screened with the Williams primer set. Two plants from each of these seven sites were screened with Operon primer Set E. The UBC primer set was too large for such comprehensive initial screening to be undertaken and, therefore, the strategy adopted was to take two plants each from Truro and Plymouth, which represented a total of four sites chosen on the basis of widest geographical separation. Primers which appeared, on first examination, to show polymorphism were then used to screen individuals taken from all sites. Three varieties of *P. communis* were surveyed alongside *P. cordata* using a subset of the primers.

There was no reproducible variability within *P. cordata* at any of the three levels of study for 98% (126) of the primers (Figure 1). The remaining four

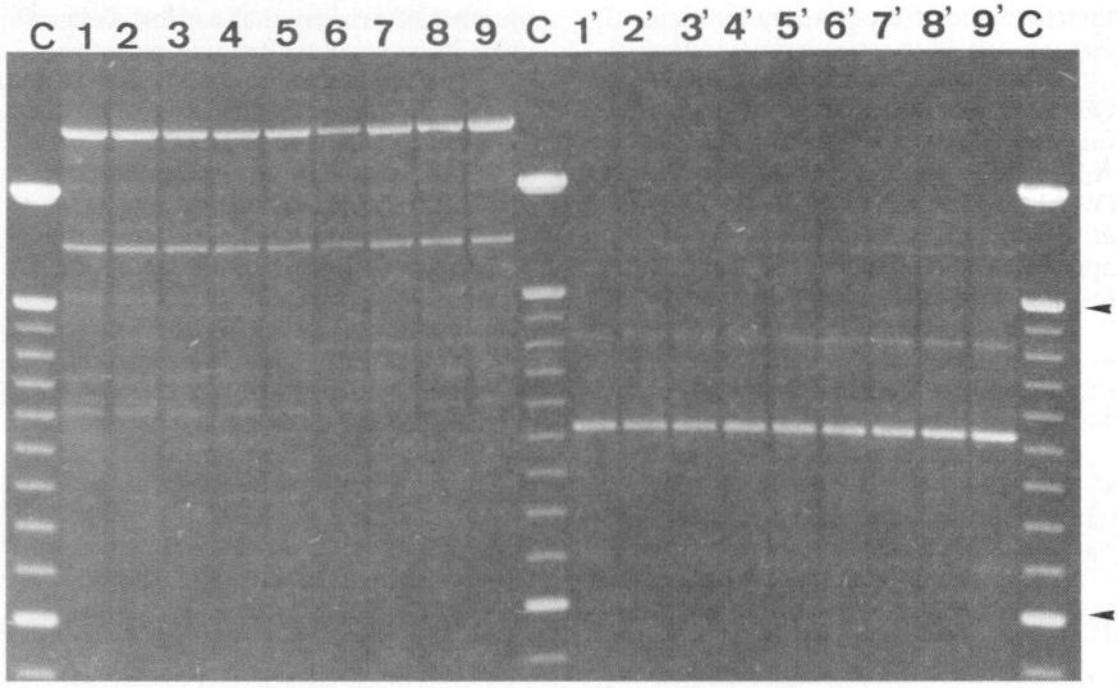

Figure 1. Sample RAPD primers showing no band polymorphism between Truro and Plymouth populations of *Pyrus cordata*.

Lanes 1–5 and 1'–5' show Truro plants from sites 1–5 respectively. Lanes 6–9 and 6'–9' show Plymouth plants from Marjon, Plymbridge, Tecalemit and Wrigleys, respectively. Lanes 1–9 show primer UBC705 and lanes 1'–9' show primer UBC710. Lanes marked 'C' contain the Gibco 100 bp DNA weight marker (600 bp and 1500 bp markers are indicated with an arrow).

(numbers 717, 756, 779, 785), all from the UBC set, detected differences, but only between the two main populations. Primer 717 showed bands at 500, 570, 660, 1000 and 1190 bp for both Plymouth and Truro populations but had a unique band at 420 bp in Truro populations and bands at 460 and 890 bp in Plymouth populations. Primer 756 showed bands unique to Plymouth trees at 700 (faint), 1650 and 1800 bp and bands unique to Truro trees at 300 and 1000 bp (Figure 2). Primer 779 showed common bands at 950 and 1900 bp, bands unique to Truro populations at 1000, 1400 and 1500 bp and bands unique to Plymouth populations at 1020, 1150 and 1550 bp. Primer 785 showed bands at 520, 780, 910, 1100, 1440 and 1500 bp with one band unique to Plymouth populations at 710 bp.

These primers were then used to compare *P. cordata* with cultivated *P. communis*. They successfully distinguished between them and ruled out the possibility of introgression of these two species in the current British populations (Figure 3).

Utilisation of the genetic data

The morphological and RAPD variation between the populations was confirmed in a crossing programme. In the first year (1994), 258 crosses between the Plymouth and Truro populations yielded 43 fruits containing 93 seeds of which 72% germinated. The British populations of *P. cordata* are, therefore, thought to consist of only two reproductively isolated clones. In order to conserve these clones and to endeavour to secure at least medium-term viability, an experimental population was designed.

In conservation genetics much debate

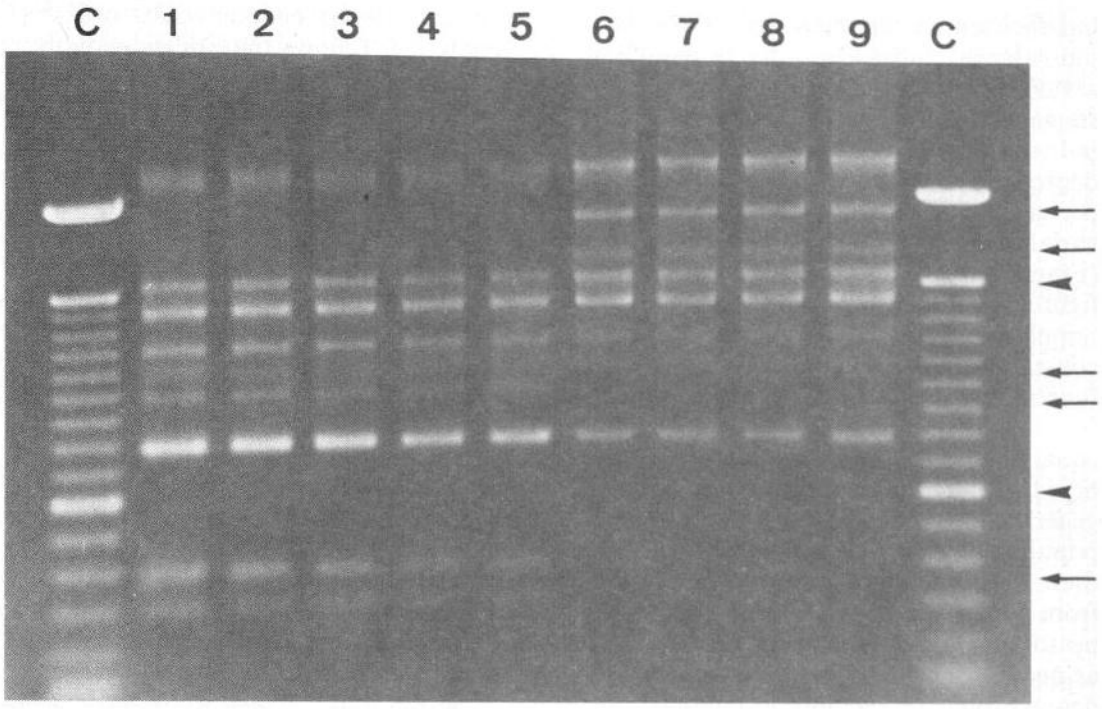

Figure 2. RAPD primer UBC756 shows band polymorphism between Truro and Plymouth populations.

Lanes 1–5 are samples from Truro sites 1–5, respectively. Lanes 6–9 are from Plymouth sites Marjon, Plymbridge, Tecalemit and Wrigleys, respectively. Lanes marked 'C' contain the Gibco 100 bp DNA weight marker (600 bp and 1500 bp markers are indicated with a short arrow ▷). Polymorphisms are indicated with a long arrow →.

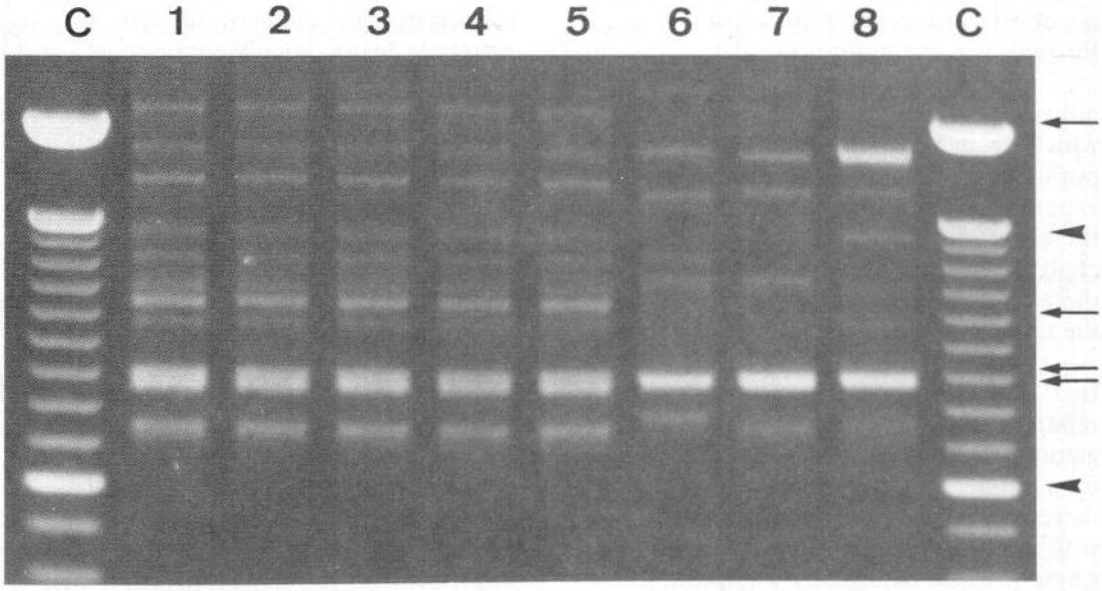

Figure 3. RAPD primer UBC724 illustrates differences found between *Pyrus cordata* and *P. communis*.

Lanes 1–3 are samples from Truro sites 1, 2 and 4, respectively. Lanes 4 and 5 are from Plymouth sites Marjon and Plymbridge, respectively. Lanes 6–8 are *P. communis* cvs Doyenne de Comice, Williams and Conference, respectively. Lanes marked 'C' contain the Gibco 100 bp DNA weight marker (600 bp and 1500 bp markers are indicated with a short arrow ▷). Polymorphisms are indicated with a long arrow →.

has focused on the minimum number of individuals required in order to maintain a viable population. It has been suggested that for most species, 50 individuals could reduce inbreeding depression to an acceptable level of 1% per generation and 500 could prevent variation being lost through genetic drift (Franklin 1980; Soulé 1980). For these figures to be relevant, the breeding population size needs to remain constant; the numbers of individuals functioning as males and females should be equal; all individuals should have an equal chance of producing offspring that breed in the next generation; and the generations should not overlap. For populations reintroduced into the wild these are difficult to control and figures from 5000 (Nunney & Campbell 1993) up to 10,000 individuals are being voiced as necessary to compensate for genetic, demographic and ecological factors (Mike Lawrence pers. comm.).

When reviewing these concepts for *P. cordata* in Britain it was noted that, between the two clones, there is a maximum of four alleles for any one gene locus and the rarest allele would have a frequency of 0.25. This assumes no novel genetic variation arising through somatic mutation. The minimum viable population figure of 50 individuals is selected to conserve alleles which occur as infrequently in a population as 5% (0.05). The experimental population design, therefore, did not focus on the typical captive breeding numbers but centred on the available allelic diversity especially at the critical *S* locus.

In *P. cordata*, as in its cultivated relatives, self-incompatibility is of the gametophytic type. This is controlled by a single *S* locus at which typically there can be many different *S* alleles within a population (S_1, S_2 etc.). If the single *S* allele carried by a haploid pollen grain matches either of the *S* alleles in the diploid genotype of the style on which it lands, that pollen grain is unable to effect fertilisation (see Richards (1986), chapter 6, for further details). It follows that all individuals must be heterozygous at the *S* locus and that a minimum of three different *S* alleles is required for a population to reproduce sexually.

A study of pollen tube growth within controlled, reciprocal interpopulation cross-pollinations was used in an attempt to assess the allelic diversity at the *S* locus. The results were inconclusive and it is still uncertain whether there are three or four *S* alleles in the two British populations. Given that the loss of one of these alleles could have a significant effect on the future reproductive performance and, therefore, the medium-term conservation of the British representatives of *P. cordata*, the experimental population was designed to accommodate this.

The experimental population

On the assumption that there are four *S* alleles in the two clones, there could be a maximum of six different *S* genotypes in a sexually reproducing population founded from both sources. From a balance of mathematical considerations (to increase the chances of each genotype being equally represented) and practical constraints, a minimum number of ten individuals of each possible *S* genotype was selected, thus indicating a minimum population size of 60 individuals.

To maximise the long-term conservation of the *S* alleles, and any other genetic diversity, it was important to ensure that all individuals would have an equal chance of contributing to the next generation. Where there are three alleles for self-incompatibility, the plants would be semicompatible with three possible offspring genotypes; however, their frequencies would vary and would be dependent on the female (Table 1 (a)). With four alleles the plants would be totally cross-compatible and there would be six possible genotypes including four offspring (Table 1 (b)).

Table 1. Segregations at the *S* locus in cross-pollinations involving three and four *S* alleles.

Parents female		male	Offspring	
(a) three *S* alleles (semicompatible crosses)				
S_1S_2	x	$[S_1]S_3$[a]	½S_1S_3	½S_2S_3
S_1S_3	x	$[S_1]S_2$	½S_1S_2	½S_2S_3
(b) four *S* alleles (fully compatible crosses)				
S_1S_2	x	S_3S_4	¼S_1S_3	¼S_1S_4
S_3S_4	x	S_1S_2	¼S_2S_3	¼S_2S_4

[a] Pollen grains carrying S_1 are unable to effect fertilisation.

In the case of three *S* alleles, each cross would yield one of the parental genotypes plus the new combination S_2S_3. If this were left unchecked 50% of a seedling-based population would consist of S_2S_3. In order to balance a newly established population, an equal number of seedlings would need to be raised from each female. In addition one-third would need to be parental genotypes propagated vegetatively. Table 2 (a) illustrates the population structure using the minimum number of 60 individuals. For four *S* alleles the expected frequencies should not vary and would not be dependent on the female. However, the parental genotypes are missing and would need to be added to balance a re-established population as illustrated in Table 2 (b). The elegance of this system is that ten vegetative propagules are required from each parent and 20 seedlings from each reciprocal cross regardless of whether there are three or four *S* alleles present.

The reintroduction

The choice of the reintroduction site was governed by a number of factors:

- the site must be within the existing range of the species;
- the site must be outside the range of likely cross-pollination with *P. communis*;
- the site must be free from disturbance;
- the climate must be similar to that of the 'native' habitat;
- the soil must be like that of the extant populations (moist and acidic).

Data on climate and soil type (Jackson 1994) were used to evaluate a shortlist of possible reintroduction sites. Planting started in September 1995. To maximise the degree of outcrossing, the planting design is of ten groups of six plants consisting of each parental genotype along with two offspring from each reciprocal cross (Figure 4). A 4 m spacing between the plants was selected to enable the crowns to intermesh to aid cross-pollination by bees. The groups of plants are 20 m apart and cross-pollination should also occur between them.

Table 2. Number of plants required for the experimental population if three or four alleles are controlling self-incompatibility.

(a) three *S* alleles					
Genotype	Vegetative from parent		Seedlings from cross		Total
	S_1S_2	S_1S_3	S_1S_2 x $[S_1]S_3$	S_1S_3 x $[S_1]S_2$	
S_1S_2	10			10	20
S_1S_3		10	10		20
S_2S_3			10	10	20
Total	10	10	20	20	**60**
(b) four *S* alleles					
Genotype	Vegetative from parent		Seedlings from cross		Total
	S_1S_2	S_3S_4	S_1S_2 x S_3S_4	S_3S_4 x S_1S_2	
S_1S_2	10				10
S_3S_4		10			10
S_1S_3			5	5	10
S_1S_4			5	5	10
S_2S_3			5	5	10
S_2S_4			5	5	10
Total	10	10	20	20	**60**

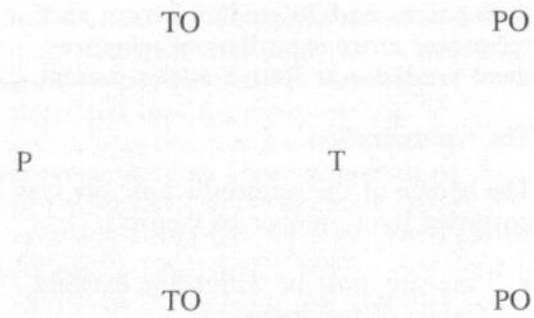

Figure 4. Planting design of a six-plant group in the experimental population (Truro parent (T), Plymouth parent (P), Truro offspring (TO) and Plymouth offspring (PO)).

Each plant will be given a unique number, its parentage recorded and they will be accurately surveyed and mapped. It is hoped that the individual DNA fingerprints of the seedling component of the population can be identified. This would enable future studies on the competitive abilities of each individual as they colonise the site through clonal and sexual reproduction.

Concluding remarks

The combination of a broad RAPD survey of individuals with test hybridisations provided both a biochemical and a biological assessment of genetic variability within *P. cordata*. The biochemically detected genetic differences and those detected on the basis of hybridisation correlate perfectly.

DNA fingerprinting proved to be an important tool for the conservation strategy. The unique information that it provided was used to:

- determine a targeted hybridisation programme which avoided wasting resources on indiscriminate pollinations;
- design the experimental population on sound genetic principles developed for this particular scenario rather than an academic application of wider principles;
- develop sexual and clonal propagation targets, which will avoid over-production and help to balance the contribution of each genotype to the next generation;
- identify the paucity of genetic variation in the British populations and to indicate the need to study diversity within mainland European populations.

Finally, it is not clear whether *P. cordata* came to Britain naturally, or was introduced long enough ago for no records to remain. If a few plants were originally imported and clonal stock planted, there would never have been much genetic variety in the British plants. Alternatively the genetic depauperacy may be a result of long-term population decline.

If the species has been introduced and propagated vegetatively, or has always been present in very low numbers, it is unlikely that any genetic adaptation to local conditions can have happened. Furthermore, with the current two founding clones, the available diversity for long-term adaptation to changes in the environment is small. A full understanding of the genetics of this species can only be gained after a similar study of mainland European populations, data from which should be available in the next five years. At that point a review should be undertaken and must include an assessment of the status of the British populations as well as the possibility of introducing additional variation from the continent.

Genetic considerations will continue to be important to the recovery programme of *P. cordata* for the foreseeable future.

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Practical aspects of threatened species management in botanic garden collections

Mike Maunder and Alastair Culham

Maunder, M., & Culham, A. 1997. Practical aspects of threatened species management in botanic garden collections. *In*: *The role of genetics in conserving small populations*, ed. by T.E. Tew, T.J. Crawford, J.W. Spencer, D.P. Stevens, M.B. Usher & J. Warren, 122–130. Peterborough, JNCC.

The practical aspects of the *ex-situ* management of threatened species in botanic gardens are reviewed in this paper. Case studies will illustrate the complexities of international species recovery projects. Island conservation projects in St. Helena and the Mascarenes are used to identify the need for *ex-situ* genetic management. Particular emphasis is given to the need for in-country facilities and training programmes. The implications of international policy and legislation, notably the Convention on Biological Diversity, are assessed.

Case studies are used to review such issues as the source and nature of breeding material and the logistics of international plant movement. An international programme for the Easter Island endemic *Sophora toromiro* illustrates the role of multidisciplinary projects incorporating a wide range of research goals. This project is attempting to restore a species now extinct in the wild. The taxonomic and genetic status of the species is ambiguous. In the long term the future of this species will depend not only upon genetic screening but ultimately on the ability of conservation managers to sustain reintroduced populations on Easter Island.

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Introduction

Traditionally European botanic gardens have developed encyclopaedic collections encompassing maximum species level diversity. Such collections represent a highly skewed sample of botanical diversity with an emphasis on taxa that are both horticulturally amenable and visually attractive. As an artefact of this curatorial policy collections within botanic gardens contain specimens of taxa that are now either threatened with extinction or 'Extinct in the Wild' (*sensu* Mace & Stuart 1994). It is becoming increasingly apparent that the effective utilisation of this material for conservation will depend on botanic gardens actively identifying conservation priorities within their collections and further developing links with *in-situ* agencies and activities (Maunder 1993). For a number of species the last individuals survive only in cultivated collections; they represent an immediate and obvious priority for botanic garden action. The management of these small and scattered populations poses a special challenge to conservation biologists.

The conservation role of botanic garden collections has been ratified in a large number of international policy documents (IUCN, UNEP & WWF 1980, 1991; WRI, IUCN & UNEP 1992; WWF & IUCN 1989). The scale of the current patterns of habitat destruction indicates that the number of threatened plant species will continue to increase beyond the present capacity of *ex-situ*

facilities to hold and manage populations. It is estimated that there are more than 1,500 botanic gardens world-wide. However, there are major imbalances in global distribution. Europe has 532 botanic gardens, Africa 82 and South America 66 (World Conservation Monitoring Centre 1992). These botanic gardens are thought to hold between 12,000 and 15,000 threatened plant species (WRI, IUCN & UNEP 1992). The majority of these samples consist of single accessions containing a single individual or, at best, a few individual genotypes.

The current status of botanic garden collections

Only in recent years have collections been developed *de novo* with the primary aim of supporting species recovery programmes. The vast proportion of threatened species within botanic gardens have been introduced to collections as components of the encyclopaedic collections reflecting taxonomic diversity or as specific subjects for research or teaching purposes. Most of these scattered individuals have little or no associated data on provenance, identity or conservation value. A number of taxa have been lost from cultivation after the extinction of the wild populations. For instance, the last specimens of both the Mauritian endemic *Asteria rosea* and the climbing Philip Island glory pea *Streblorrhiza speciosa* were lost from British collections during the 19th century. In contrast other taxa have been maintained in cultivation while their wild populations have been lost entirely (see Table 1 for examples). A number of these have been maintained for considerable periods of time as ornamentals or curiosities. For instance, the camellia relative *Franklinia alatamaha* has been in cultivation since about 1765, though it became extinct in the wild in the USA shortly after 1803.

At least 19 taxa (categorised as 'Extinct in the Wild', *sensu* Mace & Stuart 1994) are maintained in cultivation in the UK (Table 1); a large proportion of these taxa (9/19) are not managed in UK botanic gardens for conservation. Only 12 are the subjects of action or recovery plans in their country of origin. Nine taxa are the subject of international programmes linking a UK botanic garden with the country of origin. Only five (*Tecophilaea cyanocrocus, Lysimachia minoricensis, Erica verticillata, Cosmos atrosanguineus* and *Tulipa sprengeri*) will involve or have involved the reintroduction or repatriation of stock from British botanic gardens.

Many of these species are derived from very small founder stocks. For the two bulb species (*Tecophilaea cyanocrocus* and *Tulipa sprengeri*) the cultivated stocks are descended from mass wild collections that caused the extinction of these single site endemics; for *Lysimachia minoricensis* it is likely that the cultivated stock is descended from one founder individual. The cultivated stock of the cycad *Encephalartos woodii* is derived as clonal propagules from one wild founder individual.

A proportion of these species is available in the commercial horticultural trade (Erhardt & Erhardt 1990; Pereire & Bonduel 1992), most notably *Lysimachia minoricensis*, which is available from 16 retail suppliers (Lord 1994). Numerically, the largest proportion of this species is grown by commercial horticulturists. The distribution of genetic diversity is currently being investigated by us. For a small number of threatened species (such as *Tecophilaea cyanocrocus* and *Gasteria baylissiana*, the latter being a rare taxon but not yet extinct in the wild) a number of potentially important individuals, some representing wild-collected genotypes, is in private hands. For ten taxa, there is a lack of data on the status of the species in the wild, so the 'Extinct' categorisation must be treated with some suspicion. *Anthurium leuconeurum*, for instance, may be of hybrid origin (T. Croat pers. comm.).

Table 1. Taxa 'Extinct in the Wild' which are cultivated in British botanic gardens (status as recorded by IUCN/WCMC)

Taxon	No. of UK collections	Country of origin	Status confirmed	International conservation programmes	In-country conservation activities
1. *Anthurium leuconeurum*	1	Mexico	?	?	?
2. *Arctostaphylos uva-ursi* ssp. *loebreweri*	3	USA	propagated in USA	no	yes
3. *Bromus verticillatus*[a]	4	UK	yes	yes	?
4. *Calandrinia feltonii*[a]	3	Falkland Islands	yes	RBG Kew and Falklands Govt.	yes
5. *Ceratozamia hildae*	14	Central America	?	no	no
6. *Commidendrum rotundifolium*[a]	1	St. Helena	re-introduced into the wild	no	yes
7. *Cosmos atrosanguineus*[a]	5	Mexico	yes	RBG Kew with UNAM, Mexico	UNAM, Mexico
8. *Erica verticillata*[a]	10	RSA	propagated and re-introduced	UK material repatriated	yes
9. *Encephalartos woodii*[a]	7	RSA	confirmed extinct, secure in cultivation	no	yes
10. *Franklinia alatamaha*[a]	22	USA	confirmed extinct, secure in cultivation	no	yes
11. *Graptopetalum bellum*	2	Mexico	?	?	?
12. *Helichrysum selaginoides*	1	Tasmania	?	?	?
13. *Lysimachia minoricensis*[a]	10	Minorca	re-introduced by Brest BG & others	Brest BG & Medio Ambiente, Spain	yes
14. *Opuntia lindheimeri*	1	Mexico?	?	?	?
15. *Paphiopedilum delelenatii*	3		?	?	?
16. *Sophora toromiro*[a]	3	Easter Island	confirmed extinct	Toromiro Mgt. Group	yes
17. *Tecophilaea cyanocrocus*[a]	2	Chile	confirmed extinct by Chilean auth.	RBG Kew and CONAF	yes
18. *Trochetiopsis erthroxylon*[a]	3	St. Helena	re-introduced	University of Oxford	yes
19. *Tulipa sprengeri*[a]	>10	Turkey	?	RBG Kew	no

([a] = status verified by in-country authorities)

Infra-specific diversity held within collections

The collection manager is faced with many practical constraints that must be tackled during any conservation project. Stocks of 'Critically Endangered' or 'Extinct in the Wild' taxa (*sensu* Mace & Stuart 1994) held within botanic gardens are characterised by a number of shared genetic and demographic features, namely:

- highly reduced or extinct wild population;
- numerically small and/or closely related founder stocks for cultivated populations;
- fluctuating cultivated population;
- little or no associated ecological or biological information;
- poorly documented cultivation history, often no satisfactory horticultural protocols;

- stock scattered through a number of collections with varying horticultural or curatorial capacity;
- long history in cultivation, often decades or several generations;
- few co-ordinated conservation programmes involving country of origin;
- open to artificial selection, genetic drift, inbreeding and hybridisation with congenerics.

A review of the holdings within the Alpine Unit's collection at Royal Botanic Gardens, Kew, illustrates the problem of restricted representation within individual taxa (Table 2).

Table 2. Taxonomic diversity within a botanic garden collection

Taxonomic diversity	
Number of accessions	10,620
Number of families	168
Number of genera	860
Number of sub-generic taxa	4,200
Number of threatened sub-generic taxa	525
Mean number of accessions for all threatened taxa	1.6

Management of threatened species within botanic gardens

Prior to the initiation of a conservation programme a botanic garden must invest time and resources in information gathering. Currently there is no existing protocol to guide 'northern' botanic gardens on how they should manage their international conservation programmes. The Conservation Projects Development Unit (CPDU) of the Royal Botanic Gardens, Kew, is charged with identifying the conservation priorities within RBG Kew's living collections. It is currently developing a hierarchical protocol to allow collection managers to identify and subsequently develop their own conservation programmes.

Conservation Assessment and Management Plan (CAMP) workshops have been widely adopted by the zoological community to facilitate information collection and the subsequent management of threatened taxa (Seal, Foose & Ellis 1994; Ebenhard 1995). They provide a rational and comprehensive means of assessing priorities. The workshops bring together experts from a variety of backgrounds to evaluate the status of and threats to taxa within a taxonomic group or defined geographical area. Initial management recommendations are made using spreadsheets and a Taxon Data Sheet for each taxon under review. A CAMP workshop was used by RBG Kew to assess conservation priorities for St. Helena (Maunder *et al.* 1994; Ellis & Seal 1995), with other workshops being developed for India and Kenya. This allows botanic garden staff to identify potential candidate taxa for *ex-situ* management. Accordingly, material already in cultivation can be utilised or collections made *de novo*.

A series of 'Conservation Audits' has been undertaken, cross-referencing the existing IUCN Categories of Threat supplied by the World Conservation Monitoring Centre (WCMC) with the Kew accessions and supplemented with available published sources and other sources of information, for instance CAMP proceedings. This acts as a first 'cut' allowing provisional identification of conservation priorities.

Where geographical or taxonomic clusters of threatened taxa have been identified this is followed by a more detailed survey undertaken in collaboration with the appropriate taxonomic and in-country authorities. For instance, a 'Conservation Review' of the genus *Saintpaulia* (Eastwood & Maunder 1995) was undertaken in collaboration with the National Museums of Kenya, and a review of Kew's Mauritian collections was undertaken in collaboration with the Mauritian Wildlife Appeal Fund (Eastwood *et al.* 1995). These reports summarise the wild status of taxa, identify the origin, location and value of the Kew material and identify potential collaborators in-country. Importantly

they also serve to repatriate information to conservation professionals. Where possible such reports are based on field data.

Project design and development

The development of an international conservation project is not only an exercise in genetics but also, and perhaps at times more importantly, an exercise in balancing differing cultural perspectives and political objectives (Clarke, Reading & Clarke 1994; Trompenaars 1994). It has been argued (Schaller 1992) that "conservation problems are social and economic, not scientific. . .". Consequently, not only does an international recovery plan require participation from a variety of professional disciplines but also by definition from a number of nationalities and cultures.

The participation of in-country collaborators is imperative. It ensures integration with in-country legal and management authorities. It also allows the incorporation of local expertise that can guide project development. For instance, a collaborative conservation programme for the woodland understorey shrub *Abeliophyllum distichum* has been established between the CPDU, The University of Reading and Yeungnam University, Korea. Subsequent field work with Korean collaborators (Kim & Maunder 1995) has resulted in the discovery of large new populations. Accordingly the need for *ex-situ* management has been largely removed. In contrast, field work by CONAF, Chile, as part of a collaborative programme for the geophyte *Tecophilaea cyanocrocus* has confirmed its status 'Extinct in the Wild'. CONAF is managing the *in-situ* component of the project and preparing for the reintroduction of cultivated stock from RBG Kew.

Species recovery and management groups

The conservation management of a threatened taxon involves a number of stages – namely verification of wild and cultivated status, formulation of propagation and recovery plan with required collaborators, location of cultivated stocks, collection of research materials for genetic screening and subsequent utilisation – leading to distribution and repatriation of stock. The initial salvage of material from scattered collections is both time consuming and logistically demanding.

The Easter Island endemic tree *Sophora toromiro* is now 'Extinct in the Wild' and only survives in cultivation. The last surviving wild specimen was chopped down for firewood in 1960. The current status of the species in cultivation is confused by a lack of documentation for cultivated plants in Europe, Australia and Chile (Table 3).

Located 'toromiro' trees are scattered within at least 18 collections in eight countries. Initial morphological and documentary evidence suggests that nine collections are growing genuine *Sophora toromiro* trees. Only one collection (Goteborg) has documented wild origin trees. In fact, at least five collections have received stock, directly and indirectly, from Goteborg. This stock is derived from one pod, collected in 1958, probably the result of a self fertilisation event on the last surviving wild tree. Three collections are growing mis-identified stock; this material has been utilised, albeit unsuccessfully, for reintroductions on at least two occasions.

A programme of genetic screening has been instigated by us using RAPDs and multi-locus microsatellites to assess surviving diversity within the species. Initial results have confirmed the existence of true toromiro trees in cultivation in the USA, Chile and Australia. The screening is aimed at both identification of taxa and assessing the level of genetic diversity within the target species.

Table 3. Status and distribution of *Sophora toromiro* cultivated stocks

Collection	Origin	Identity	Accession notes
1. Christchurch, NZ	?	Prob. *Sophora microphylla*	none
2. Bonn BG, Germany	Goteborg ?	*Sophora toromiro*	none
3. Goteborg, Sweden	Easter Island via Thor Heyerdahl	*Sophora toromiro*	yes
4. Vina del Mar, Chile	?	*Sophora toromiro*	none
5. [a]Pablo Pitze, Chile	?	?	none
6.[a]Behn, Chile	?	*Sophora toromiro*	none
7. [a]Sudsuki, Chile	?	?	none
8. [a]Ingerson, Chile	?	?	none
9. Melbourne, Royal Botanic Gardens, Australia	?	*Sophora toromiro*	none
10. Brest, France	Christchurch, NZ	Prob. *Sophora microphylla*	yes
11. Edinburgh, UK	Christchurch, NZ	Prob. *Sophora microphylla*	yes
12. Missouri, USA	Viña del Mar	?	yes
13. Kew, UK	Goteborg	*Sophora toromiro*	yes
14. Frankfurt, Germany	Goteborg and Bonn	*Sophora toromiro*	yes
15. Waimea, Hawaii	Missouri	?	yes
16. Les Cedres, France	Goteborg and Bonn	*Sophora toromiro*	yes
17. Menton, France	Goteborg and Bonn	*Sophora toromiro*	yes
18. [a]Schick, Chile	?	?	?

[a] = name of private householders cultivating specimens of *Sophora toromiro*.

The future survival of *Sophora toromiro* depends on securing the existing stock in cultivation, locating and assessing surviving genetic diversity, developing a propagation programme utilising this diversity and repatriating material prior to a reintroduction on Easter Island. Accordingly a multidisciplinary research team has been established to manage the programme, the Toromiro Management Group.

Collection and transport of plant material

The initiation of a propagation and conservation programme will involve the location and collection of specimens from a number of botanic gardens. For instance a recovery programme for the endemic tree *Hibiscus liliiflorus* from Rodrigues involved stock from collections in Hawaii, Mauritius, Switzerland, Denmark, France, Japan and the UK. The wild population of this small tree is reduced to two individuals with no natural regeneration. It has become evident that a large proportion of the cultivated stock is wrongly identified. A propagation programme for the 'Critically Endangered' Hawaiian endemic *Alsinidendron* sp. (Maunder *et al.* 1995b) has involved stock from France, Germany, Denmark and the UK. The increasingly strict legal (e.g. CITES) and phytosanitary restrictions are making the international movement of stock more difficult than in the past. For instance the fear of Lethal/Yellowing Disease is making it increasingly difficult to move cocoid palm material within the tropics and similar restrictions operate in the Caribbean region for Zingiberaceae so as to protect the cut-flower trade.

Field programmes on St. Helena and Mauritius

The UK Dependent Territory of St. Helena lies in the South Atlantic Ocean about 500 nautical miles to the east of the mid-Atlantic ridge. A large proportion of the island's endemic flowering plants are facing a high risk of

extinction in the near future (three taxa 'Extinct in the Wild', nine taxa 'Critically Endangered' and two taxa 'Endangered'). Eight species have recovered to the status of 'Conservation Dependent' following management (Maunder 1995), though the remaining risks are still many (Table 4).

Table 4. Summary statistics on St. Helenan endemic plant taxa (derived from Maunder 1995)

Status	Number of taxa
Requiring intensive conservation management	13
Wild populations of fewer than 50 individuals	4
Critically Endangered taxa with no stock in cultivation	4
Heavily reduced or no natural regeneration	5
Largest proportion (>80%) of the population at one site	4
Pathogens impacting on adult mortality levels	4
Contamination of local genotypes through planting	3
Relictual populations/individuals surviving outside of natural vegetation	3
Very low or heavily reduced seed fertility	5
Threatened by introduced invasive weeds	11
Known extinctions	7

To develop conservation management on the island it was first necessary to review the conservation infrastructure. There is little point in developing a detailed species conservation programme if this is undermined by government policy and land-use/resource conflicts. With the International Institute for Environment and Development (IIED), a Sustainable Environment and Development Strategy was initiated (RBG Kew & IIED 1993). This reviewed prevailing and potential environmental issues and made recommendations on mechanisms for natural resource management on the island (Maunder *et al.* 1995a). This project has expanded to encompass soil erosion and rehabilitation studies, integrated pest management, recovery planning for endemic plants and fisheries management. Island government decision-making has been changed as new committees have been established to provide a cross-disciplinary perspective on natural resources.

The future of the endemic flora rests ultimately upon effective habitat management and the development of propagation skills and facilities on the island to support genetic management (Maunder 1995). The rapid loss of potential founders from already small and severely depleted wild populations places great emphasis on the development of horticultural salvage skills. Once the stock has been salvaged the genetic screening can proceed. In particular, field gene banks are required to hold large breeding stocks to expand the population out of numerical bottlenecks quickly and mitigate against any drift effects.

St. Helena is not alone in requiring an urgent investment in practical horticultural management. The Indian Ocean island of Mauritius has a large number of plant species at dangerously small populations. For instance, there are 14 species reduced to a single wild individual, 38 species are known from 2–10 individuals, 52 species are known from 11–50 individuals, and 39 species have a wild population between 51 and 250 (Wayne Page, RBG Kew & Mauritian Wildlife Appeal Fund, unpublished data). The sheer number of threatened species (104 species with populations less than 50) precludes an initial programme of comprehensive genetic screening and management for all species. Emphasis will need to be placed on direct *in-situ* habitat management to ensure the survival of the majority of species, with the development of supporting *ex-situ* facilities.

Conclusions

The Convention on Biological Diversity (Glowka, Burhenne-Guilmin & Synge 1994) makes several references to species management which will influence the future development of international projects. Importantly, these include the requirement to develop *ex-situ* facilities in the country of origin (Article 9a), collaborative training and research

programmes (Article 12a, b & c), and the recognition that states have sovereign rights over their biodiversity and that access to those resources will all have to be negotiated *a priori* (Article 15).

The successful conservation of threatened plant species in botanic gardens is restricted by a chronic lack of information to guide curators and collection managers on the selection of target species. Any resulting programme for a priority species must first ensure that it is firmly linked to the in-country authorities and that it is based on all the available founder individuals. This process of salvage from geographically scattered collections can take years. Genetic screening will be necessary to assess the nature and distribution of genetic diversity in poorly documented and scattered botanic garden collections. Single species propagation programmes by northern botanic gardens should be viewed only as components of a programme designed to support in-country activities and facilities.

The retention of taxa now 'Extinct in the Wild' within botanic gardens is indicative of botanic gardens' abilities to maintain horticulturally amenable stock over time. The surviving cultivated stocks of such taxa, in all probability, represent a small proportion of the founder stock's genetic diversity. To progress from preservation by accession number to population management of such taxa, botanic gardens need to adopt species genetic management at a much earlier stage and record details of individuals rather than grouped accessions.

Botanic gardens need to recognise that long-term conservation success depends on the strengthening of local skills and facilities. Prior to embarking on single species programmes the national or local infrastructure should be reviewed, and any propagation programme must be firmly linked to the needs of the relevant authorities in the country of origin. The initial priorities of a species programme should be two-fold: firstly the development of appropriate facilities; and secondly the urgent location and propagation of available founders.

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Section 4 – Conservation genetics and demography

The papers in this section explore the interaction between population genetics and population ecology from a conservation perspective and illustrate, once again, the importance of genetic variation for the planning and implementation of conservation policy and practice.

Michelle Tremayne and John Richards present a second empirical study of the Scottish primrose. The main threat to the populations of this rare flowering plant, endemic to Scotland, is habitat destruction. Nevertheless, they conclude that outcrossing increases both vegetative and reproductive performance and that artificially mediated cross pollination may be worth consideration as a means of increasing population vigour.

One of the key debates in conservation surrounds the relative contribution of genetic factors (such as inbreeding depression and genetic drift) and demographic factors to the local extinction of a species. Joop Ouborg and Rob van Treuren suggest that such distinctions are artificial, and that inbreeding depression and demography are interacting, rather than separate, processes. Whilst it may be that genetic problems are secondary to those imposed by environmental stochasticity and demography, the latter effects are likely to be much larger on populations already under genetical stress. Theoretical models using demographic and genetic data show that the effect of inbreeding depression on the probability of extinction is very dependent on where in the life-cycle the depression occurs and which stages of the life-cycle contribute most to population growth.

Mike Gillman and Jonathan Silvertown step back from practical conservation management and look at how population demography and population genetics influence the quantitative assessment of the conservation status of a species. Such quantitative assessments are increasingly used by international organisations such as IUCN to assign conservation value to species and thereby to prioritise international conservation initiatives. Gillman and Silvertown note, in particular, the sensitivity of these analyses to the sampling time period, and urge caution in precipitate answers based on short time-scales.

This section of the proceedings is completed with three short papers, presented in poster format at the meeting. Joyce Schoondergang finds evidence for both negative (inbreeding depression) and positive (purging of deleterious alleles) effects of inbreeding in the small scabious, endangered in The Netherlands. Odilia Velterop also studied a rare Dutch plant, in which pollen exchange by bumble bees dictates the balance between selfing and outcrossing. Gabriel Nève studied the genetic structure of a rare butterfly in Belgium and found a classic metapopulation structure which has several implications for practical management of the species. Taken as a group, these three posters serve to emphasise the importance of understanding genetic effects when managing small populations of plants and animals. The dispersal of genetic material between geographically separated populations can maintain heterozygosity, however that dispersal is achieved.

The effects of breeding system and seed weight on plant fitness in *Primula scotica* Hooker

Michelle Tremayne and A.J. Richards

Tremayne, M., & Richards, A.J. 1997. The effects of breeding system and seed weight on plant fitness in *Primula scotica* Hooker. *In*: *The role of genetics in conserving small populations*, ed. by T.E. Tew, T.J. Crawford, J.W. Spencer, D.P. Stevens, M.B. Usher & J. Warren, 133–142. Peterborough, JNCC.

The rare Scottish endemic *Primula scotica* is threatened with extinction in its few remaining sites. This is mainly because of habitat destruction, but also low rates of flowering and hence low seed production within populations. We tested the hypothesis that progeny fitness may be affected by the breeding system and/or seed weight.

Surveys of three populations during the course of three years revealed an inverse association between seed number and mean seed weight per capsule, suggesting that seeds compete for a limited maternal resource.

Experiments under controlled conditions established that heavier seeds (>60µg) germinate earlier than lighter seeds (<40µg), and that seedlings from heavy seeds produce significantly heavier plants at both four and eight weeks. However, the breeding system only affected performance at the germination stage when seeds weighing >60µg resulting from outcrosses germinated earlier than similar seeds originating from selfs.

Plants derived from seed of both outcrossed and selfed origins were transplanted back into the site of seed origin in 1994. Results after one year indicate that outcrossing may confer a fitness advantage; leaf number per plant and rosette diameter are significantly larger in individuals resulting from outcrossing, and although flowering rates were low (<4% of survivors), all flowering individuals were from outcrossed origin. In contrast, results from transplants into the same area using plants raised from known seed weights revealed that although seed weight effects were present at the time of transplantation, any size advantage of plants derived from heavier seeds had disappeared within the first year.

We conclude that under natural, stressed conditions, initial seed weight of an individual has little effect on subsequent long-term plant performance. However, outcrossing does increase both the vegetative and flowering performance of offspring. This could have a significant effect on the seed production of outcrossed individuals. The relevance of these results to the conservation of this species is discussed.

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Introduction

Primula scotica Hooker, a hexaploid (2n = 6x = 54) homostyle member of *Primula* section *Aleuritia* Duby (Bruun 1932), is a rosette-forming perennial species. It is one of Britain's few endemic vascular plants and has a very restricted distribution, occurring only in suitable habitats (coastal pasture, machair and sedge heath) along the north coast of Scotland from Cape Wrath to Wick and on some westerly islands in the Orkney group (Bullard *et al.* 1987). The number of individuals within populations varies from the hundreds to the tens of thousands; however, the total number of sites is rapidly declining and the species is threatened with extinction in many of its remaining sites. Like many other plant species threatened with extinction, the main cause is habitat destruction via human activities (Falk & Holsinger 1991), in this case resulting from agricultural improvement of the land (Berry 1985) and to a certain extent increasing pressure from tourism in the area. This ongoing loss of the already restricted habitats of *P. scotica* is compounded by increased mortality during cold winters and the low flowering rates and hence low seed production of many individuals (Bullard *et al.* 1987). Low fecundity is a serious impediment to recruitment into populations as the only method of reproduction in this species is by seed.

Flowering occurs during two periods, early and late summer; some individuals produce flowers in both periods, others only in one. At each flowering two to six deep pink/purple flowers are borne on a single scape. After pollination and fertilisation these mature into multi-seeded capsules. The homostylous flowers suggest that seed-set in *P. scotica* is primarily by within-flower selfing (Ritchie 1954) unlike many other *Primula* species. However, seed can also be set by outcrossing events. When assessing how reproduction by seed may affect subsequent progeny fitness, two factors should be considered: the breeding system involved (selfing or outcrossing), and the weight of the individual seed itself.

Selfing can lead to inbreeding depression, the significant reduction in offspring fitness caused by inbreeding. Inbreeding depression is thought to be caused primarily by the expression of deleterious recessive alleles (Charlesworth & Charlesworth 1987), and can be expressed at different life history stages (Kalisz 1989). However, the level of inbreeding depression expressed within populations is affected by complex interactions between mating system, effective population size and the history of inbreeding (Charlesworth & Charlesworth 1987). *Primula scotica* populations, which are predominantly selfing and have a long history of inbreeding, may therefore exhibit low levels of inbreeding depression because of the purging of recessive deleterious alleles (Barrett & Charlesworth 1991). In a complex allopolyploid such as *P. scotica*, fixed heterozygosity can and does occur (Glover & Abbott 1995) so that some replicated loci may be protected from homozygosity. However, although polyploidy *per se* may also buffer against homozygosity, a recent RAPD and isozyme survey revealed no genetic polymorphism in this species (Glover & Abbott 1995). Nevertheless, increased fitness of outcrossed, compared to selfed, progeny because of heterotic effects may still be expected as a result of the crossing of differentially fixed lines (Charlesworth & Charlesworth 1987). There is equivocal evidence for this effect in *P. scotica*; results of a 16-year study from a population on Orkney (Bullard *et al.* 1987) reveal some individuals to be more highly vigorous and fecund than are the majority of the others, leading to the formation of two distinct subpopulations. Two explanations were suggested by the authors. First, some individuals may be more vigorous because of chance arrival into different quality microsites. Secondly, fitter

individuals may have arisen from outcrossing events in suitable weather conditions and be more vigorous for heterotic reasons.

Within multi-seeded fruits, negative relationships between seed number and seed weight have sometimes been found (Wolf *et al.* 1986; Lyons & Antonovics 1991). Such trade-offs are thought to arise from competition between individual seeds for a limited maternal resource and have been reported for many species including *P. farinosa*, an outcrossing relative of *P. scotica* (Baker, Richards & Tremayne 1994). Greater weight of an individual seed has sometimes been found to affect subsequent plant fitness positively in both seedling and adult stages. Heavier seeds typically germinate earlier and more reliably and produce faster growing seedlings (Stanton 1985; Baker, Richards & Tremayne 1994). This larger juvenile size may enhance (i) survival into adulthood (Schaal 1980), (ii) adult size and (iii) fecundity (Mazer 1987).

The high seed-set per capsule expected from selfing in *P. scotica* may cause low individual seed weight. However, if seed-set within a capsule is reduced, the resultant low seed-set would lead to the formation of fewer but heavier seeds, which may in turn give rise to fitter individuals compared to those arising from lighter seeds. Also, if some of the seeds are set by outcrossing events, the resultant progeny should show an increased fitness over selfed progeny because of heterotic effects.

The aims of this study are (i) to investigate the amount of variation in seed production present in populations of *P. scotica,* and (ii) to assess the relative importance of breeding system and seed weight to plant fitness at the seedling and adult stages. Ideally, this latter work should be carried out under natural conditions. However, the prohibitively small seed size makes field experiments to assess seedling fitness impossible. Therefore, early life history stages were assessed under controlled growth conditions, whereas long-term effects on adult plants were investigated by transplants into wild populations.

Materials and methods

Seed analysis

Seeds were collected from each of four populations as outlined in Table 1. For populations at Balnakeil and Strathy Point, Sutherland, the numbers of seeds in two capsules per scape were counted, whereas all capsules sent from the Orkney populations were included in the analysis. Twenty seeds per capsule were individually weighed on a Mettler MT5 micro-balance to provide a mean weight per capsule. Only capsules containing set seed were subsequently used for data analysis.

Table 1. Details of study populations and sample collection for *Primula scotica.*

Site and year sampled	Grid reference	Sampling procedure
North coast of Scotland		
Balnakeil 1992 (BK92)	NC387688	1 scape from 10 plants
Balnakeil 1993 (BK93)	NC387688	1 scape from 20 plants
Balnakeil 1994 (BK94)	NC387688	1 scape from 20 plants
Strathy Point 1993 (SP93)	NC829695	1 scape from 20 plants
Orkney		
Hill of Borwick 1992 (HB92)	HY222167	1 capsule from 20 plants
Yesnaby 1992 (YB92)	HY222160	1 capsule from 20 plants

Effect of breeding system and seed weight on germination and seedling growth

In 1993 flowering plants raised from wild-collected Balnakeil seed were hand-pollinated to produce seed of outcrossed and selfed origins. Three flowers from each of ten different maternal plants were selfed and then emasculated. To simulate outcrossing three flowers from each of ten different maternal plants were emasculated and pollinated using pollen from one of ten paternal plants. Selfed and outcrossed seeds were each

bulked and weighed into two seed-weight classes (>60μg and <40μg), 100 seeds in each. Each of the four classes of seed was split to provide equal numbers of plants for dry-weight harvesting at four and eight weeks postgermination. Seeds were sown onto damp filter paper in Petri dishes and chilled at 4°C in the dark for four weeks. To study germination, Petri dishes were transferred to a greenhouse at a temperature of 24°C, with a 12 h day. Germination was scored at the appearance of cotyledons and was noted every other day for 42 days. After germination, seedlings were pricked out into plastic module trays (each module = 27 cm^3) containing John Innes no.1 compost and allowed to grow on until harvested at either four or eight weeks. Harvested plants were washed, dried at 30°C for seven days and weighed.

Effect of breeding system and seed weight on adult plant fitness in natural populations

Two separate transplant experiments were undertaken at Balnakeil, one to investigate breeding system effects and one to investigate the influence of seed weight. In the first, hand pollinations using different maternal and paternal parents were performed to produce seed of outcrossed and selfed origins as outlined above. After maturation, seeds were collected and to control for any possible seed weight effects, only seeds weighing 51–70 μg were selected. For the second, seeds of known weights ranging from 20 to 120 μg were randomly selected from wild-collected Balnakeil seed. Plants for both experiments were raised as outlined previously and after four months were transplanted back into the Balnakeil population along one of four transect lines. The position of each plant was marked *in situ* with a plastic stick, and also by its position along the transect. Immediately prior to transplantation (August 1994), each plant was measured for three vegetative performance indicators: number of leaves per plant, rosette diameter, and number of new rosettes produced. It should be noted that although transplants were the same age as any newly recruited seedlings at the time of transplantation, they were larger in size. However, no comparisons were made between transplants and wild plants in this study. Transplants were revisited in July 1995 and those surviving were measured for the same vegetative characters and for evidence of flowering.

Table 2. Seed analysis for all populations of *Primula scotica* sampled.

Site *n* = no. caps. with seed	Seed no./cap. Range Mean (± SE)	Seed wt./cap. (μg) Range Mean (± SE)	Correlation coefficient	% capsules with <60 seeds
BK 92 (*n* = 20)	20–246 146.05 (± 12.9)	30.5–100.9 57.87 (± 3.82)	–0.602 **	10
BK 93 (*n* = 39)	3–159 66.65 (± 8)	7.6–98.4 38.67 (± 2.46)	–0.673 ***	37.5
BK 94 (*n* = 38)	4–161 116.38 (± 6.53)	39–100.2 46.72 (± 3.19)	–0.433 **	25
SP 93 (*n* = 33)	4–152 64.53 (± 7.05)	12.9–97.6 36.41 (± 2.42)	–0.006 NS	25
HB 92 (*n* = 20)	63–176 118.15 (± 6.83)	24.6–81.5 44.92 (± 2.83)	–0.293 NS	0
YB 92 (*n* = 20)	81–206 130.15 (± 7.46)	32.1–91.7 69.57 (± 2.24)	–0.317 NS	6

Significance: $P < 0.05$ *; $P < 0.01$ **; $P < 0.001$ ***.

Results

Seed analysis (Table 2)

Within individual capsules, seed-set varies from 3 to 246 and mean seed weight from 7.6 to 100.9 µg. Considerable variation both between populations and within Balnakiel between years is also shown, with the highest mean seed-set (146) being seen at Balnakeil in 1992 and the lowest occurring at both study sites in 1993. Populations at Balnakeil (in each year), Hill of Borwick and Yesnaby show a negative association between seed weight and seed number per capsule (r = –0.293 to –0.673). However, correlations are significant only when 10% or more of the capsules sampled contain fewer than 60 seeds, as was found for the population at Balnakeil in each of the three years. Although the population at Strathy Point had 25% of capsules with fewer than 60 seeds, the correlation is not significant. Upon examination, 56% of the capsules sampled from this location were found to have a fungal infection affecting the seeds. Where capsules were heavily infected causing late abortion, their seeds were counted as unset. This decreased the seed set for these capsules. However, they were probably still being resourced by the maternal plant late into maturation so that unaffected seeds would not benefit from reduced seed number.

Effect of breeding system and seed weight on germination and seedling growth

Germination scores of seeds allocated to four- and eight-week harvests were pooled to give a germination sample size of n = 100 for each breeding system at each weight class. Breeding system only had a significant effect on mean number of days to germination for seeds weighing more than 60 µg, where crossed seeds germinated earlier than did selfed seeds (Table 3). However, seed weight has a large positive effect on germination, with heavy seeds (>60 µg) from both crossed and selfed origins germinating significantly earlier than those weighing less than 40 µg (Table 3). Final germination totals at 42 days varied from 78 to 100. χ^2 analysis on seeds bulked by weight class reveals that lighter seeds have a decreased chance of germination (χ^2_1 = 34.28, $P < 0.001$). When bulked by breeding system, no significant difference is found (χ^2_1 = 0.025). However, further tests outside this experimental programme suggest that all seeds weighing more than 10 µg will eventually germinate.

No significant differences in mean seedling dry weight between crossed and selfed individuals were found for either heavy or light seed weights at 4 weeks and for heavy seed weights at 8 weeks (Figure 1). For seeds weighing less than 40 µg, individuals of outcrossed origin were significantly smaller compared to

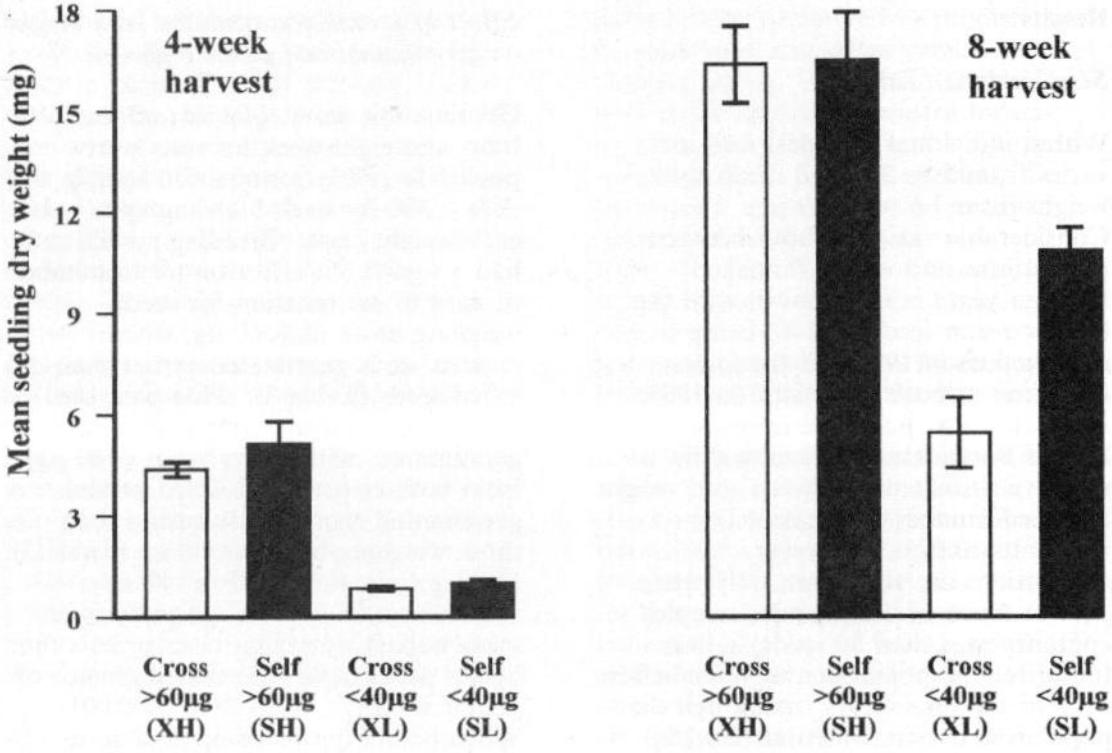
Table 3. Number of days to germination and total number of germinating seeds (out of 100) after 42 days for *Primula scotica* seeds of outcrossed and selfed origins in two seed weight classes.

Seed class (n = 100)		Mean wt. of sample, µg (± SE)	Mean no. days to germination (± SE)	No. seeds germinated after 42 days
Cross > 60 µg	(XH)	64.42 (± 1.04)	8.02 (± 0.29)	96
Cross < 40 µg	(XL)	29.74 (± 1.10)	13.98 (± 0.39)	81
Self > 60 µg	(SH)	66.37 (± 0.94)	9.09 (± 0.33)	100
Self < 40 µg	(SL)	39.78 (± 0.90)	13.36 (± 0.43)	78

t-tests on mean number of days to germination

Effect of breeding system	Effect of seed weight
XH vs SH: t_{190} = 2.44*	XH vs XL: t_{152} = 12.32****
XL vs SL: t_{155} = 1.07 NS	SH vs SL: t_{154} = 7.91 ****

Significance: $P < 0.05$ *; $P < 0.01$ **; $P < 0.001$ ***; $P < 0.0001$ ****.



t-tests	4 weeks	8 weeks
XH vs XL	$t_{46} = 15.3$, $P < 0.0001$	$t_{61} = 7.52$, $P < 0.0001$
SH vs SL	$t_{51} = 6.49$, $P < 0.0001$	$t_{61} = 3.53$, $P < 0.001$
XH vs SH	$t_{61} = 1.02$, NS	$t_{79} = 0.05$, NS
XL vs SL	$t_{59} = 1.36$, NS	$t_{43} = 4.32$, $P < 0.0001$

Figure 1. Mean dry weight (mg ± SE) of *Primula scotica* seedlings at 4 and 8 weeks postgermination.

those from selfed when harvested at 8 weeks (Figure 1); however, many of the former were inadvertently subjected to drought during the course of the experiment which reduced growth. Seed weight has a large positive effect on mean seedling dry weight (Figure 1). At both 4 and 8 weeks, seedlings from heavy seeds were significantly heavier than those originating from light seeds.

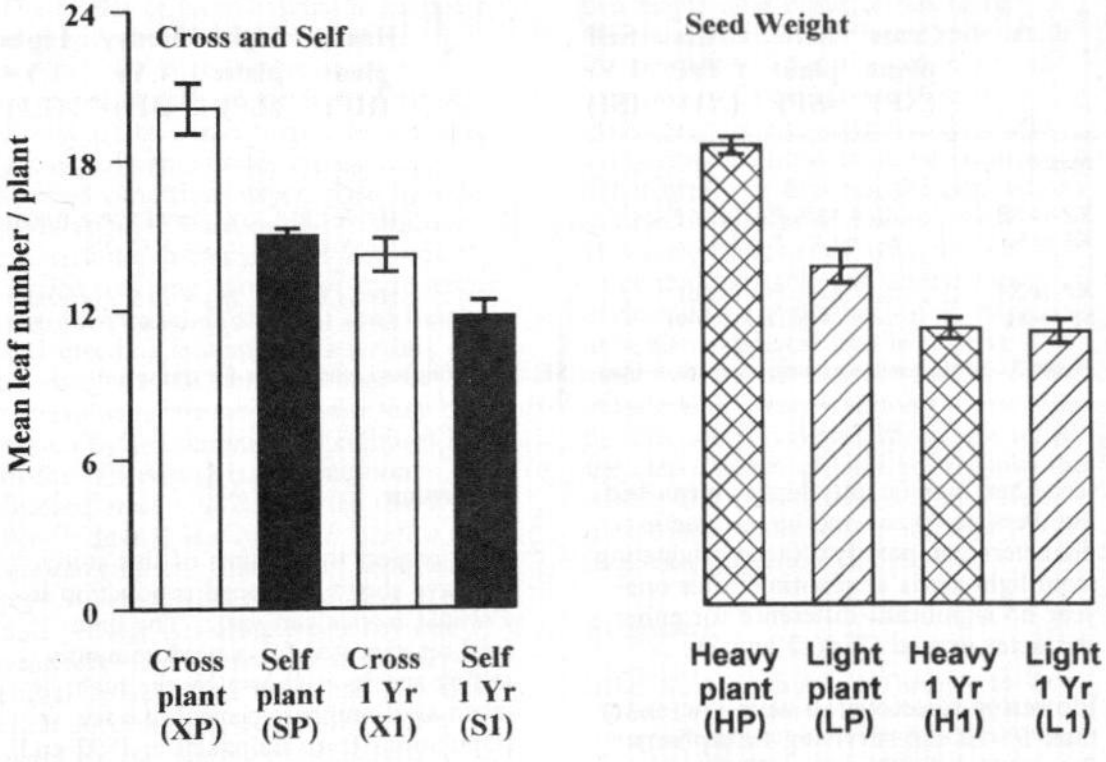
Effect of breeding system and seed weight on adult plant fitness

All positions of transplants were relocated after one year by either the presence of a plant, or a plastic marker stick if the plant had not survived. Overall, the percentage of transplants surviving after one year is high (83%). Although χ^2 analysis (Table 4) reveals no differential survival between plants of selfed or outcrossed origins, plants raised from light seeds had a slightly better chance of survival after one year compared to those from heavier seeds. Whereas surviving individuals of both transplant experiments had significantly fewer leaves per plant and smaller rosette diameters after one year (Figures 2 and 3), there were also differences within the two experiments. Breeding system had significant effects on vegetative growth characters, with plants of outcrossed origin having significantly higher mean leaf numbers per plant and larger mean rosette diameters per plant compared to selfed at planting and after one year (Figures 2 and 3). In contrast, although plants originating from heavy

Table 4. Number of *Primula scotica* plants surviving and the number of survivors flowering one year after transplantation for plants raised from seeds of a) selfed and outcrossed origins, and b) known seed weights.

Transplant type (no. planted)	No. surviving	No. survivors flowering
a) Cross (n = 50)	45	8
Self (n = 50)	41	0
Statistical test	χ^2_1 = 1.33 NS	Fisher's exact P = 0.006 **
b) > 61 µg (n = 94)	71	0
< 60 µg (n = 76)	67	2
Statistical test	χ^2_1 = 4.38 *	Fisher's exact P = 0.24 NS

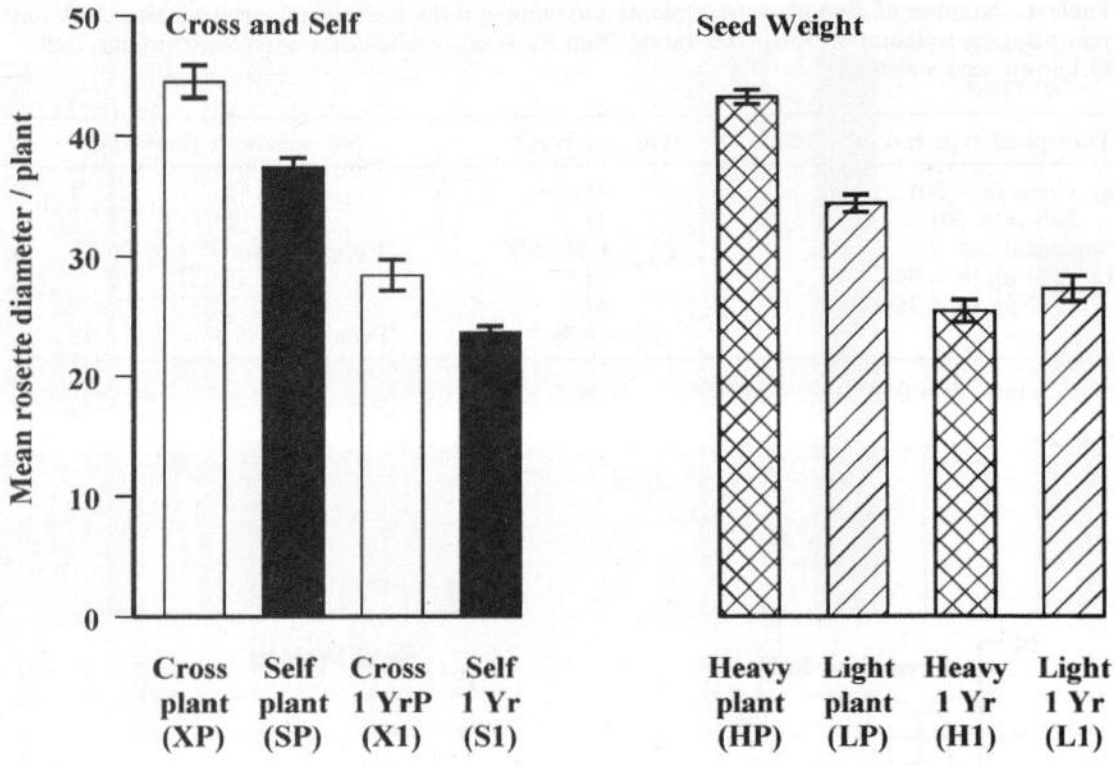
Significance: $P < 0.05$ *; $P < 0.01$ **.



t-tests

XP vs SP	t_{56} = 4.87, $P < 0.0001$	HP vs LP	t_{166} = 10.52, $P < 0.0001$
X1 vs S1	t_{80} = 2.66, $P < 0.01$	H1 vs L1	t_{132} = 0.20, NS
XP vs X1	t_{82} = 4.84, $P < 0.0001$	HP vs H1	t_{150} = 13.42, $P < 0.0001$
SP vs S1	t_{55} = 4.67, $P < 0.0001$	LP vs L1	t_{112} = 4.68, $P < 0.0001$

Figure 2. Mean leaf numbers per plant (± SE) at planting and after 1 year for transplant experiments with *Primula scotica* investigating the effect of breeding system and seed weight.



t-tests

XP vs SP	$t_{76} = 4.58$, $P < 0.0001$	HP vs LP	$t_{153} = 9.63$, $P < 0.0001$
X1 vs S1	$t_{55} = 3.42$, $P < 0.01$	H1 vs L1	$t_{132} = 1.32$, NS
XP vs X1	$t_{89} = 8.61$, $P < 0.0001$	HP vs H1	$t_{122} = 16.5$, $P < 0.0001$
SP vs S1	$t_{85} = 14.84$, $P < 0.0001$	LP vs L1	$t_{116} = 5.46$, $P < 0.0001$

Figure 3. Mean rosette diameters per plant (mm ± SE) at planting and after 1 year for transplant experiments with *Primula scotica* investigating the effect of breeding system and seed weight.

seeds had significantly higher mean leaf numbers per plant and larger rosette diameters compared to those originating from light seeds at planting, after one year no significant difference for either character existed (Figs. 2 and 3).

Flowering rates overall were low; fewer than 5% of the surviving transplants flowered in the first year (Table 4). Fisher's exact test of independence revealed that breeding system did affect flowering with significantly more plants of outcrossed origin flowering compared to those from selfed origins. However, no significant difference between plants raised from heavy and light seed was found (Table 4.).

Discussion

With respect to the aims of this study, we have shown that seed production in *Primula scotica* can vary. The high seed-set expected for a predominantly selfing species is shown by the high mean seed numbers per capsule for populations from Balnakeil in 1992 and 1994, and from both Orkney populations. However, the low mean seed numbers for populations at Balnakeil and Strathy Point sampled in 1993 demonstrate that seed-set can be reduced. These lower values are similar to those reported for the outcrossing relative *P. farinosa* where seed-set is limited by pollinator activity (Baker, Richards & Tremayne 1994). Trade-offs

between seed number and mean seed weight per capsule create variations in seed weight.

Seed weight has a large positive effect on germination and early seedling vegetative growth under controlled growth conditions. In contrast, with the exception of germination of heavy seeds, our study in these conditions revealed no advantage to seeds from outcrossed origin compared to those from selfing for either number of days to germination or seedling vegetative growth. Possibly, heterotic effects are not always apparent in early life-history stages or when grown under glass in non-competitive conditions (Wolfe 1993; Hamilton & Mitchell-Olds 1994).

The fitness of an individual is gauged by its ability to survive and produce progeny which themselves have an increased chance of survival. Fitness effects are best investigated by carrying out experiments under the natural stressed conditions experienced by wild populations. Monitoring of both our transplant experiments has not been carried out long enough for us to assess any differential effects that seed weight and breeding system may have on survival. In this perennial species, survival early in the first year may be biased by the remnants of compost left in the soil during transplantation. Bullard *et al.* (1987) reported that flowering and survival in *P. scotica* are size-dependent. The loss of significant seed weight effects on vegetative growth and the low flowering frequency one year after transplantation show that the initial weight of an individual may have little effect on subsequent long-term plant fitness, despite possible large effects at the seedling stage. However, the results from transplants derived from seed of outcrossed and selfed origins reveal that outcrossing increases both the vegetative and reproductive performance and could therefore have a significant effect on long-term plant fitness as hypothesised by Bullard *et al.*(1987).

Is the fact that outcrossed individuals are more fit relevant to the conservation of this species? Unfortunately for *P. scotica* the opportunities for outcrossing appear to be at best infrequent. First, if Bullard *et al.* (1987) are correct in assigning low flowering rates to inbreeding depression, as also suggested by our study, then repeated selfing has a positive feedback element attached to it. Selfing will produce fewer flowering individuals hence decreasing the potential number of crossing partners. This in turn leads to a higher chance of selfing and the loop is repeated. Secondly, climatic conditions are notoriously poor in this region, so that pollinator activity may be very low during flowering time. And lastly, even if pollen transfer between two plants does occur, it has to be between genetically different individuals for any fitness advantage arising from heterosis to be expressed. Recent evidence from an isozyme and RAPD study revealed little or no variation either within or between the populations sampled. (Glover & Abbott 1995). However, Ennos *et al.* (this volume) show that heritable variability does occur within populations of *P. scotica*, as is also suggested by the relative vigour of our outcrossed seedlings. We suggest that a conservation programme for this species might address not only the conservation of habitat, but also the raising and transplanting of artificially outcrossed families in an attempt to increase population vigour.

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Inbreeding depression, environmental stochasticity and population extinction in plants

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An ongoing discussion in conservation biology is focussed on the relative importance of genetic erosion and inbreeding depression, in comparison to demography and environmental stochasticity, for the probability of extinction of small, isolated populations. In this paper the view is supported that inbreeding depression and demography are interacting rather than separate entities.

This view is illustrated with data from research on the threatened perennial *Scabiosa columbaria*. It is demonstrated that small populations of this species in The Netherlands contain less genetic diversity than large populations. Despite this evidence for genetic erosion no differentiation in fitness or inbreeding depression between small and large populations could be demonstrated.

A density-independent, stage-structured demographic model was built to assess the potential impact of inbreeding depression on both population growth rate and probabilities of extinction of two small and two large populations. Demographic data were collected from each of the four populations in three consecutive years, and inbreeding depression in the same populations was measured in greenhouse experiments.

Analyses of the model showed that the effect of inbreeding depression on both the population growth rate and the probability of extinction was very dependent on (1) whether inbreeding depression occurred early or late in the life-cycle, (2) on whether early or late stages of the life cycle contributed most to the population growth, but (3) especially on the interaction of these two factors. Building on this result, and based on the general patterns that have been described for the distribution of inbreeding depression across stages and on the distribution of the relative contribution of different life stages to the population growth rate, it is argued that inbreeding depression will enhance the probability of extinction in small, isolated populations of outcrossing species that have recently gone through a bottleneck.

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Introduction

One of the main objectives of conservation biology is to reach an understanding of the relative importance of the various factors that may influence the extinction of populations and species. The fields of taxonomy, population biology, ecology and population genetics all approach extinction while referring to their own paradigms, so it is conservation biology that is challenged to take a multidisciplinary approach to integrate the different views into an overall concept.

Population extinction has both deterministic and stochastic causes. Deterministic extinction is the ultimate consequence of a death rate per individual that is on average higher than the per capita birth rate, which is characteristic of situations where the population is occurring in non-optimal or marginal habitats, for instance because of habitat degradation, pollution or habitat destruction. Inventories of extinct and threatened species suggest that the majority of present day extinctions have deterministic causes (Primack 1995).

In contrast, stochastic influences on population dynamics will result in a non-zero probability of extinction even for populations that grow, i.e. that have, on average, a higher birth rate than death rate. This probability is determined by the size of the population and by the mean and variance of the population growth rate (Lande & Orzack 1988), such that the smaller the population, and the lower the mean growth rate and the higher its variance, the higher the probability of extinction.

Mean and variance of the population growth rate are a function of the value of the 'vital rates', the birth and death processes, in the population. Causes of variation of the vital rates can be categorised as being demographic, environmental and genetic (Shaffer 1981; Gilpin & Soulé 1986). Demographic stochasticity, the variation among individuals in their probability of dying and in the number of offspring they produce, will in small populations cause fluctuations of the population size, which will increase the probability of extinction (MacArthur & Wilson 1967; Richter-Dyn & Goel 1972). In larger populations the individual variation will average out. This is the reason why demographic stochasticity is important only in very small populations. Environmental stochasticity, the random variation in the biotic and abiotic environment, which is affecting all individuals in the population equally, will also lead to fluctuations in population size and increases in extinction probabilities. Models, in which the impact of both demographic and environmental stochasticity on extinction are evaluated, in general show that the latter has substantial effects on extinction even at moderately high population sizes (Shaffer 1987; Menges 1992). Environmental stochasticity and natural catastrophes (Mangel & Tier 1994) are regarded as the primary stochastic threats to populations.

Third, in small, isolated populations genetic drift and inbreeding will cause a decline of genetic diversity and an increase in homozygosity, a process known as genetic erosion (Ouborg 1993a; Van Treuren 1993). While the loss of genetic diversity may reduce the evolutionary potential of the population to adapt to changing environments, the increased homozygosity may result in an immediate reduction of individual fitness through inbreeding depression (Frankel & Soulé 1981). Inbreeding depression reduces the average birth rate and increases the average death rate, thereby increasing the extinction probability of the population.

Much attention, both theoretical and experimental, has been given to the genetic problems of small populations. However, it has been argued that genetic problems may often be secondary to the

problems imposed by environmental stochasticity and demography and that extinction may occur before genetic problems become evident (Lande 1988; Menges 1991a). Nevertheless, it can be envisaged that environmental stochasticity will have a much larger effect on populations already affected by inbreeding. Therefore it is clear that the relative importance of inbreeding depression and environmental stochasticity can only be evaluated in demographic models that incorporate both.

Inbreeding depression in plants

Inbreeding depression, the reduction of fitness in offspring from matings among relatives, is a widespread phenomenon in higher plants. A large number of studies have dealt with the evolution of selfing in plants, and the selective force that inbreeding depression imposes on that evolution (Charlesworth & Charlesworth 1987). However, few studies of inbreeding depression have been conducted with reference to differences in population size or extinction probabilities. Several characteristics of inbreeding depression may pertain to its relevance for extinction of small plant populations.

In plants, inbreeding depression has almost without exception been assessed by comparing offspring of selfings with offspring of random outcrossings. There is, however, little evidence for a direct relationship between population size and outcrossing rate. Only if a reduction in population size is associated with a reduction in population density may the outcrossing rate decrease (Antonovics & Levin 1980; Farris & Mitton 1984; Murawski & Hamrick 1991; Van Treuren *et al.* 1993a). Inbreeding in small populations is therefore of a different nature. In small populations the likelihood of any two alleles being identical by descent is higher than in a large population. Therefore, even in a randomly outcrossing small population the inbreeding level will rise, according to the formula:

$$F_t = 1 - (1 - \tfrac{1}{2}N_e)^t$$

where F_t is the present inbreeding level, N_e is the effective population size and t is the number of generations. (Effective population size is a value corrected for unequal sex ratio, for variation in reproductive output among individuals, etc. In general the effective population size is (much) smaller than the counted number of reproducing individuals in the population). Under strict selfing F_t will increase from 0 to 0.5 in one generation. However, in a randomly outcrossing population with $N_e = 40$, a similar increase would take more than 50 generations. Therefore, inbreeding depression in small populations may often be less severe than suggested by the literature on selfing.

Part of the debate on the relevance of inbreeding for extinction of small populations concentrates on the role of selection. There is increasing evidence that inbreeding depression is caused by the expression in homozygous state of deleterious recessive alleles (Charlesworth & Charlesworth 1987; Johnston & Schoen 1995). Selection will fairly rapidly reduce the frequency of these alleles in the population, a process known as purging (Barrett & Charlesworth 1991). It has been argued that problems of inbreeding depression are temporary and small populations with a long inbreeding history will not exhibit inbreeding depression. Recently, however, others have argued that much of the inbreeding depression may be caused by many alleles with small deleterious effects (Lande 1993), which are difficult, if not impossible, to purge. Recent evidence suggests that inbreeding depression in the late stages of plant life cycles may at least partly be caused by alleles with small effects (Husband & Schemske 1996).

In mating system evolution it is the effect of inbreeding on life time

reproductive success that is the critical parameter. However, when concerned with the effect of inbreeding on extinction it may be more important to take the distribution of inbreeding depression over the various life stages into account. For instance, Mills & Smouse (1994) demonstrated in their demographic model that extinction was affected more by survival depression than by fecundity depression. Husband & Schemske (in press), in a review of the inbreeding literature on plants, showed that inbreeding depression is non-uniformly distributed across the life stages. Moreover, inbreeding depression may not be correlated between life stages in the same species (Ouborg & Van Treuren 1994).

A number of inbreeding depression studies in plants have demonstrated significant variation among families in inbreeding depression (Dudash 1990; Ågren & Schemske 1993; Eckert & Barrett 1994; Ouborg & van Treuren 1994; Parker, Nakamura & Schemske 1995). Genetic variation in inbreeding depression may even be more general than reported, because most studies were not designed to investigate among-family differences. Within population variation in inbreeding depression is a prerequisite for purging (Barrett & Charlesworth 1991; Johnston & Schoen 1994) and may be caused by genetic drift and/or variation in the inbreeding level within the population (Pray & Goodnight 1995). Within-population variation in inbreeding depression may also influence the probability of extinction, as is shown below.

Inbreeding depression in plants is reported to be environmentally dependent. Higher inbreeding depression has been found under more stressful environmental conditions, where stress may be expressed in terms of low light levels (Schemske 1983), density (e.g. Schmitt & Ehrhardt 1990), (sib-) competition level (Koelewijn 1993), or as a contrast between greenhouse and the more stressful natural environment (e.g. Dudash 1990). As a consequence inbreeding depression in the field could vary with spatial and/or temporal variation in environmental conditions. Therefore, evaluating the relevance of inbreeding to the extinction process should be directed at measuring the synergistic effect of inbreeding depression and environmental stochasticity rather than measuring the two effects separately.

Genetic erosion in *Scabiosa columbaria*

Small scabious *Scabiosa columbaria* L. is a perennial plant of dry, calcareous grasslands along the big rivers and in the chalk grasslands in the south of The Netherlands. It is a short-lived species, with an estimated half-live of adult cohorts ranging from 2 to 5 years (Ouborg 1993a). Between 1956 and 1988 80% of the *S. columbaria* populations along the Rhine system in The Netherlands became extinct, while only a few new sites were colonised (Ouborg 1993b). In 1990 the species was designated as Most Vulnerable in the Red Data List of Dutch plants (Weeda, van der Meyden & Bakker 1990).

Seeds germinate exclusively in spring and seedlings emerge from March till May (Schenkeveld & Verkaar 1984). Adults flower from early August till late November. Rosettes may produce several flower stalks which have one or more flower heads, each with 40–100 flowers. Each flower produces a fruit which contains one seed. Seeds are predominantly dispersed by wind within a few metres of the maternal plant, making genetic exchange between isolated populations highly unlikely (Verkaar, Schenkeveld & van de Klashorst 1983).

The demography of the species is strongly influenced by the density of the surrounding vegetation. In dense vegetation with lower light intensities, germination is lower, shoot growth of seedlings is reduced (Verkaar &

Schenkeveld 1984a), and mortality of seedlings is higher (Verkaar & Schenkeveld 1984c). Flowering and seed production are increased at higher nutrient levels and light intensities (Verkaar & Schenkeveld 1984b).

The flowers are pollinated by insects. Altough individual flowers are protandrous, with the pollen being emitted 2–3 days before stigmas are receptive, there is considerable overlap in timing of male and female functions within flowerheads. Nevertheless, estimations of outcrossing rates in four populations yielded values that were very close to 1 (Van Treuren *et al.* 1994). Inbreeding in this species must therefore be biparental.

In a survey of 12 populations, using 12 isozyme loci, a significant correlation between population size and both proportion of polymorphic loci and number of alleles per locus was found (Van Treuren *et al.* 1991). The genetic differentiation among small populations (n = 7) was more than twice as high as among large populations (n = 5) (G_{ST} = 0.236 vs G_{ST} = 0.101 respectively), suggesting high levels of genetic drift and low levels of gene flow among the small populations (Van Treuren *et al.* 1991). In addition, a significant correlation between population size and the level of phenotypic variation in morphology and life history was found (Ouborg, Van Treuren & Van Damme 1991). The combined results strongly suggested that the small populations of this species were affected by genetic erosion.

In an inbreeding experiment, with individuals from small and large populations, severe inbreeding depression in biomass production, root development, adult survival, seed set and overall fitness was found. No such inbreeding depression was found in germination success, seedling to adult survival, flowering percentage and number of flowerheads (Van Treuren *et al.* 1993b). Thus, *Scabiosa columbaria* is highly susceptible to inbreeding, as was expected from the high outcrossing rates found in these populations (Van Treuren *et al.* 1994). There was no correlation between population size and either fitness or inbreeding depression, despite the difference in genetic diversity among the populations. There was also no correlation between population size and the effects of interpopulation crossing. Such an effect would be expected, based on the idea that interpopulation crosses would restore heterozygosity and genetic diversity in small but not in large populations.

These results demonstrate that reduced genetic diversity, as measured at marker loci, is not necessarily associated with reduced fitness. The same conclusion was also drawn from similar research on *Salvia pratensis*, another threatened perennial in The Netherlands (Ouborg & Van Treuren 1994, 1995).

Relatively little is known about fitness differences among large and small populations of the same species. Lower seed set was found in small, compared to large, populations of *Dianthus deltoides* (Jennersten 1988), *Eupatorium resinosum* (Byers & Meagher 1992) and *Senecio integrifolius* (Widén 1993). Reduced germination percentages in small populations of *Silene regia* (Menges 1991b) and *Ipomopsis aggregata* (Heschel & Paige 1995), but not in *Senecio integrifolius* (Widén 1993), were reported. No effect of population size on the investigated fitness components was found for *Salvia pratensis* (Ouborg & Van Treuren 1995). It is clear that there is an urgent need for data on the relationship between population size and fitness.

Demographic modelling

Although there were no consistent fitness differences between small and large populations, the inbreeding experiments demonstrated that significant inbreeding load was present in all populations, including the small ones. This indicates

that if the populations remain small, inbreeding depression may become a problem in the future when populations become more inbred. A demographic modelling approach was used to determine the potential impact of inbreeding depression on the growth rate and probability of extinction of two small and two large *Scabiosa columbaria* populations.

The life cycle of *Scabiosa columbaria* was described in a density-independent matrix projection model with discrete time-steps (Figure 1a). The model had the following important characteristics.

(1) Individuals were categorised into five stages: seeds, seedlings, juveniles, vegetative adults and reproductive adults. The model describes the route an individual may follow through the life cycle, in terms of transition probabilities, i.e. the probabilities of survival to the next stage and the number of seeds produced per reproductive individual (Figure 1a).

(2) To incorporate inbreeding depression in the model, reproductive adults produced two types of seed. Any reproductive individual produced either outcrossed (non-inbred) seeds or inbred seeds. The outcrossed seeds follow the basic life-cycle, with the basic probabilities of transition. The inbred seeds follow a life-cycle where the probabilities of transition are affected by a stage-specific inbreeding depression value (Figure 1b).

(3) The model was first analysed in a time-invariant form, i.e. where all transition probabilities were constant in time. Analysis of this time-invariant model with standard mathematical techniques results in values for both the population growth rate and the relative contribution that each transition makes to this growth rate (Van Groenendael, de Kroon & Caswell 1988; Caswell 1989). This latter term, the relative

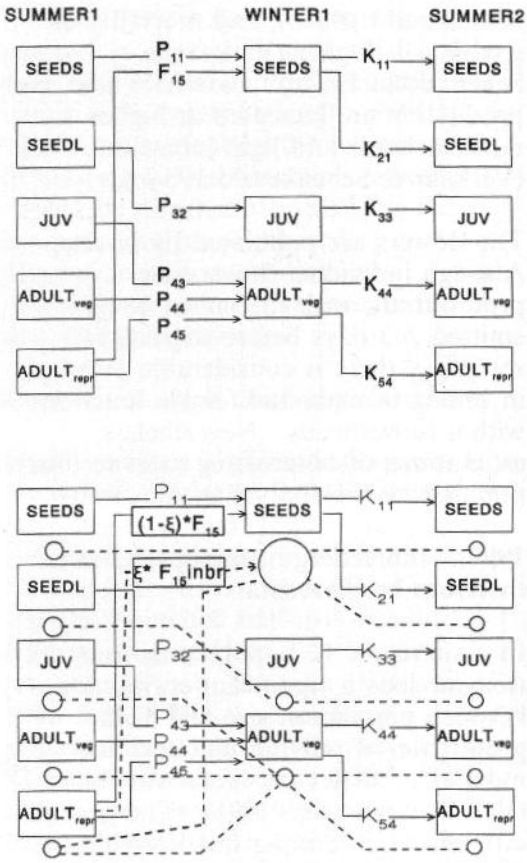

Figure 1. The life cycle flow chart of *Scabiosa columbaria* without (a, upper diagram) and with (b, lower diagram) inbreeding depression incorporated. In the lower flow chart a constant fraction ξ of the seeds each year starts a cycle of individuals that are affected by inbreeding (circles connected by dotted lines), while a fraction $(1 - \xi)$ enters the non-inbred cycle (square boxes connected by uninterrupted lines).

contribution of a transition to to the population growth rate, is generally referred to as "the elasticity of the transition" (De Kroon *et al.* 1986) and can be calculated for each transition in the model.

The model was also analysed in the stochastic form, i.e. transition values were variable in time. This analysis produced a mean and variance for the population growth rate. Together with the population size this mean and variance define the probability of population extinction and the expected time to extinction.

Table 1. Summary of the analyses of the time-invariant model for the two large (WRAK, OLST) and the two small (RUIT, KDYK) populations. For each population the growth rate is given. Only the WRAK population has a positive growth rate, indicating that it is increasing in size over time. Furthermore, for each population the left column (ELAS) gives the relative contribution that the corresponding stage made to the population growth rate, i.e. the elasticity of the stage (note that the elasticities sum to one for each population). The right column (RP) gives a measure of inbreeding depression in each stage (RP is relative performance of selfed progeny compared with outcrossed progeny). Both elasticity and relative performance vary among transitions within populations and among populations for each transition. Most importantly, the distribution of both parameters differs among populations (see text).

	WRAK		OLST		RUIT		KDYK	
population growth rate	0.196		−0.073		−0.251		−0.108	
	ELAS	*RP*	*ELAS*	*RP*	*ELAS*	*RP*	*ELAS*	*RP*
reproductive output								
x germination	0.176	1.06	0.064	0.70	0.033	0.96	0.094	1.13
seedling survival	0.176	1.04	0.064	0.85	0.033	1.14	0.094	0.92
juvenile survival	0.096	1.08	0.036	0.72	0.018	1.31	0.024	0.84
adult survival	0.259	0.65	0.463	0.16	0.471	0.26	0.180	0.53
adult flowering	0.293	1.02	0.374	1.14	0.445	0.58	0.608	0.89

In summary, this model takes account of differences in inbreeding depression across life stages, and of variance in inbreeding depression among families within the population. However, inbreeding depression values are constant in the model and thus not affected by purging or by accumulation of inbreeding (Halley & Manasse 1993; Mills & Smouse 1994). Therefore the model gives insight into the interaction between demography and inbreeding depression on a short time-scale.

In a three-year field study in two small (KDYK, RUIT) and two large (WRAK, OLST) populations the transition values, i.e. the survival and fecundity values, were estimated for each population separately (Ouborg 1993a). From greenhouse inbreeding studies on the same four populations, for each population the stage-specific inbreeding depression values were estimated (Table 1; Van Treuren *et al.* 1993b).

The results of the analysis of the basic model, i.e. the time-invariant model without inbreeding depression, are summarised in table 1. In the three-year observation period, only the large WRAK population was increasing in size. If these three years are representative of the future demography, the other three populations are drifting towards extinction. The negative growth rates in these populations coincide with relatively high vegetation biomass production suggesting that extinction would at least partly have deterministic causes.

Table 1 also shows that the elasticities of the different stages were variable both among stages within populations and among populations within stages. Table 1 further demonstrated that inbreeding depression varied in a similar fashion among stages and populations. Thus for each population there were specific combinations of elasticities and inbreeding depression across the life cycle.

Incorporating inbreeding in the model decreased the growth rate of the large WRAK population and, to a lesser extent, that of the small KDYK population, but had little effect on the growth rate of the other two populations (Figure 2). This difference was even more pronounced when environmental stochasticity was introduced into the model, by random sampling transition values from the three-year field study. These differences between populations were reflected in the effect inbreeding had on the extinction probabilities of these populations and on their expected time to extinction (Figure 3).

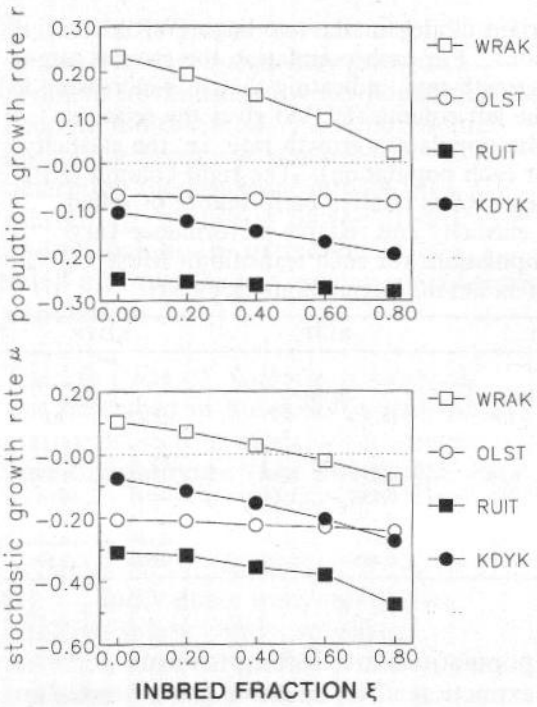

Figure 2. The population growth rate of two large (WRAK, OLST) and two small (RUIT, KDIJK) *Scabiosa* populations as a function of the fraction ξ of individuals that are affected by inbreeding. The upper graph gives the relationship in a constant environment. The lower graph gives the relationship in a fluctuating environment, i.e. where environmental stochasticity is influencing population dynamics.

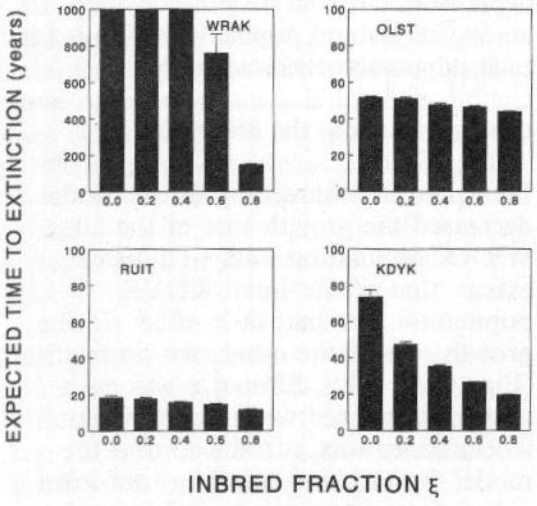

Figure 3. The expected time to extinction (ETE) for the four *Scabiosa* populations. Each value is a mean of ten runs. For the WRAK populations, below ξ = 0.6 only some runs led to extinction, while above ξ = 0.6 all runs led to extinction (see figure 2).

Discussion

Detailed analyses of these results revealed that this difference in effect of inbreeding among populations could be explained by the interaction between the distribution of inbreeding depression across life stages and the distribution of elasticities across stages. This interaction can best be understood by giving a few extreme examples.

- First, consider the example of a population with highest elasticities early in the life cycle (e.g. the germination stage), but strong inbreeding depression late in the life cycle (e.g. survival of adults). In that case inbreeding depression will have relatively little effect on the population growth rate, because it only affects stages that make a minor contribution to population growth rate.
- Next consider the reverse case, with highest elasticities late in life, but strong inbreeding depression early in life. Again, inbreeding depression will not affect the growth rate much, for identical reasons.
- Finally consider the case with highest elasticities late in life, and high inbreeding depression in all stages. Perhaps surprisingly, inbreeding depression will not affect growth rate very much. That is because inbreeding depression early in life does reduce the frequency of inbreds in the population, but does not affect growth rate at that stage. Although the high inbreeding depression in the late stages, with the high elasticities, potentially could affect growth rate very much, selection earlier in life has decreased the frequency of inbreds, so that not many of them are left to affect growth rate in the late life stages.
- Inbreeding depression will only have a large impact on population growth in those cases where high inbreeding depression and high elasticity are present in the same life

stages. An additional requirement is that, if these stages are the stages late in the life cycle, inbreeding depression should be low or absent in the early stages.

Conclusions

The results of this model indicate that the impact of inbreeding depression on extinction is apparently dependent on the distributions of inbreeding depression and elasticities across stages. Although these distributions are population-dependent, some general patterns have been described. Inbreeding depression tends to be concentrated in the late life stages in self-fertilising species, but is bimodally distributed among outcrossing species (Husband & Schemske 1996). The distribution of elasticities across stages is dependent on the population growth rate. The population growth rate is positively correlated with elasticities of early life stages and negatively with elasticities of late life stages (Silvertown *et al.* 1993; Silvertown, Franco & Menges 1996; Oostermeijer *et al.* 1996).

When plotting these patterns on the specific examples given above, inbreeding depression may contribute to extinction in two distinct categories of population (Figure 4). First, inbreeding depression may speed up extinction in declining small populations that have been small for a considerable time. Although the decline may have deterministic causes, inbreeding depression may make an additional contribution. Second, inbreeding

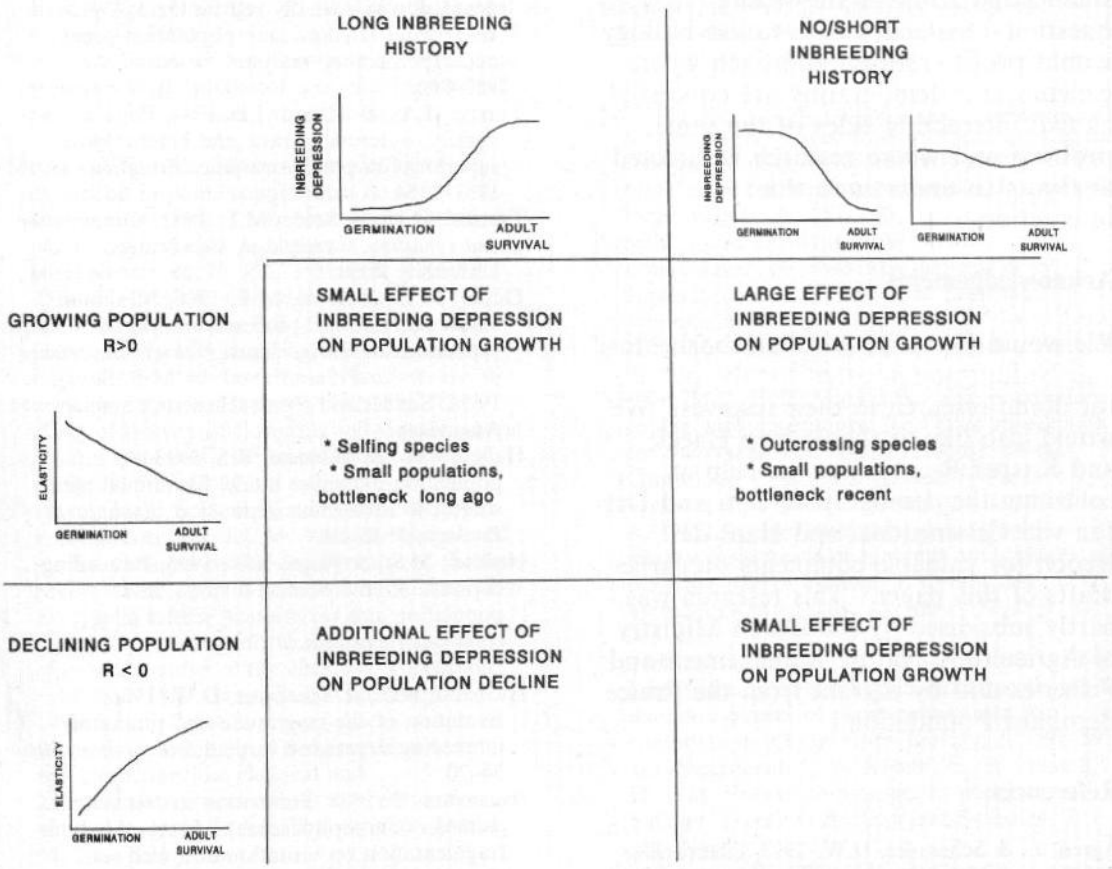

Figure 4. Scheme illustrating the general trends in the distribution of inbreeding depression and elasticities across life stages, and their interactive effect on the influence of inbreeding depression on population growth.

depression may increase the probability of extinction in growing but small populations of outcrossing species that became small recently. This effect is, however, only present for a limited number of generations, because the population will grow to a size where stochastic extinction is unlikely and/or purging will change the distribution of inbreeding depression across life stages.

Thus, under specified conditions, inbreeding depression may be an important factor in population extinction. Inbreeding depression leads to a small window in time with increased risk of extinction for small populations of outcrossing species that have recently gone through a bottleneck.

The final conclusion must be that asking whether demography or genetics is more important in the extinction of small populations is the wrong question. Instead, conservation biology would profit from an approach where genetics and demography are conceived as two interacting sides of the same problem and where research is directed at trying to understand this interaction.

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Population extinction and IUCN categories: the uncertainty of ecological measurement

M.P. Gillman and J. Silvertown

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Criteria which provide a framework allowing all species to be placed in categories of threat have recently been adopted by the IUCN council (Species Survival Commission 1994). They rely heavily on assessment of population size, trends in population change over time, spatial distribution of populations and probabilities of population extinction.

We discuss the problems of estimating some of the parameter values needed to satisfy the criteria, using examples from one animal and one plant population. In particular we question how sensitive the category placements are to the duration of measurement of relevant parameters, i.e., what is the quantity of data required in order to ensure that the species receive the appropriate management action? Four fundamental parameters are discussed: the population size, the maximum population density, trends in population size with time and the temporal variation in the rate of population change. The time to extinction is considered as a function of these parameters. The results are discussed with respect to population dynamics and population genetics.

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Introduction

> "The important point is that a mature [population] viability analysis is one that integrates all of the factors in a biologically realistic way. It is misleading to frame the issue as 'which is more important, genetics or population dynamics?' These are not isolated additive factors, though we may decide to treat them that way in the early stages of analysis." (Soulé 1987, p. 180.)

The analysis of the fate of small populations has enjoyed a boom as marked as the extinctions which it sets out to study (e.g. Soulé 1987; Pimm, Lee Jones & Diamond 1988; Thomas 1990; Foley 1994). The importance of largely theoretical studies has been acknowledged by IUCN's adoption of the new categories of threat. These categories require population data including estimation of population size, trends over time and determination of the probability of extinction (Table 1 and details below). Yet, as many people who have tried to use these criteria or who have experience of population extinction analyses have asked (e.g. Taylor 1995), are the data ready for the analysis? The aim of this paper is to cast an ecological perspective on the problem of small population size illustrated by the estimation of suitable parameters for the IUCN criteria. Whilst the emphasis here is on population dynamics, we believe there are also important lessons for population genetics.

Table 1. IUCN categories of threat with adequate data sets. See text for details of rates and sizes.

IUCN categories of threat based on adequate data sets:

Critically Endangered
Endangered
Vulnerable

Species fall into these categories if one of the following parameters is decreasing at a given rate or below a certain size:

population size
area of occupancy
extent of occupancy
probability of extinction

From a conservation perspective, small is a population size with a (given) high probability of extinction. Extinction may occur due to deterministic or stochastic processes or a combination of the two (reviewed by Ouborg & Van Treuren this volume). The stochasticity has its source in demographic stochasticity (relevant to very small populations, Ouborg & Van Treuren this volume) and/or environmental stochasticity. It is also possible that unpredictability is caused by deterministic processes (chaos) although this is likely to be rare (Ellner & Turchin 1995). With stochasticity we need to assign a probability of population extinction or an expected (mean) time to extinction (or the converse of time of persistence), given a certain initial population size. All other things being equal, the smaller the initial population size, the higher the probability of extinction or the shorter the time to extinction.

We will focus on a few key parameters, estimated from abundance data collected over time, which are central to estimates of how rapidly a population is expected to go extinct. The main parameters are (1) initial population size, (2) variance in intrinsic rate of population change (r), (3) maximum population size and (4) trends in population size with time. We will discuss 1, 2 and 3 to start with, arriving at estimates of time to extinction and then, in the light of these estimates, consider the effect of 4. The first three parameters are also central to population genetics. For example, the possibility of inbreeding depression depends on past effective population size (N_e). To estimate the distribution of values of N_e over a large number of generations we could determine the probability distribution of r from samples of variance and mean and then use that, with other appropriate parameters (calculation of N_e is described by Lande & Barrowclough (1987) and Nunney & Elam (1994)) in a population model to predict time-series of N_e. It would then be possible to predict, given current patterns of change, how frequently N_e will have dropped below critical values in the past. It would thus be possible to predict the frequency and extent of bottlenecks. Lande & Barrowclough (1987) emphasised the contribution of repeated crashes in a population to inbreeding depression, whilst a single bottleneck in a species with high r does not necessarily produce a loss of genetic variation in the long term. Ouborg & Van Treuren (this volume) support the latter view for plants. Soulé (1987) noted that r and its variance are an important point of contact between population genetics and dynamics.

Estimation of the time to extinction and relationship to IUCN criteria

Assume a population is changing in abundance according to the simple density-independent rule:

$$N_{t+1} = \lambda N_t \qquad 1.$$

where N_t is the population size or density at time t and λ is the finite rate of population change. Here population size is as recommended by Nunney & Elam (1994) for calculating effective population size, i.e., the number of potentially reproductive individuals. This is also the measure used in the

IUCN criteria. N may also represent a population vector composed of individuals of different age or stage. In this case λ is the dominant eigenvalue of the population projection matrix. Ouborg & Van Treuren (this volume) consider a stage-structured model of the plant *Scabiosa columbaria* and discuss the effect of the distribution of inbreeding depression across stages on extinction probability.

When $\lambda = 1$ the population does not change in size; when $\lambda > 1$ the population increases geometrically; and when $\lambda < 1$ the population declines asymptotically towards extinction. If λ varies from year to year due to environmental and/or demographic stochasticity then the population will fluctuate in abundance. A series of low values of λ could result in a population going locally extinct (although mathematically we would have to assume a threshold size for extinction as λ will only approach 0). Because N_t is multiplied by λ each year we take natural logs of Eqn 1 to give:

$$\ln N_{t+1} = \ln \lambda + \ln N_t \qquad 2.$$

$\ln \lambda$ (= r) is usually normally distributed and the mean and variance (v_r) can be found by standard analysis of $\ln (N_{t+1}/N_t)$. The details of assessment of probability of extinction and the validity of this model were reviewed by Foley (1994) for discrete time models and Goodman (1987) for continuous time models. We use the equation of Foley (1994), giving time to extinction (*Te*) as a function of v_r, N_0 (the initial population size) and k (the maximum population size) for a population with a mean r of 0:

$$\mathrm{Te} = (2\ln (N_0)/\mathrm{v_r}) (\ln (\mathrm{k}) - \ln (N_0)/2) \qquad 3.$$

k in this model and the continuous time model (Goodman 1987) is not an average maximum value (i.e. carrying capacity) but a strict upper boundary with the population proceeding on a random walk from N_0 bounded by k and 0 (extinction). If $N_0 = \mathrm{k}$ then Eqn 3. simplifies to:

$$\mathrm{Te} = (\ln (\mathrm{k}))^2/\mathrm{v_r} \qquad 4.$$

One of the new IUCN criteria uses the following probability of extinction levels:

Critically Endangered: 0.5 (50%) within 10 years or 3 generations, whichever is longer.
Endangered: 0.2 within 20 years or 5 generations, whichever is longer.
Vulnerable: 0.1 within 100 years.

We will focus on two short-lived species whose generation times are respectively one year and short but unknown. To use the IUCN criteria with Eqns 3 and 4 we need to convert *Te* into probability of extinction. This can be done (following Foley 1994) if extinction is viewed as a Poisson process and therefore *Te* approximated as:

$$\mathrm{Te} = -\mathrm{t}/\ln (1-\mathrm{P}) \qquad 5.$$

where P is the probability of extinction and t is the number of years over which extinction is measured.

In terms of *Te*, the IUCN thresholds are therefore:

Critically Endangered: 14.4 years
Endangered: 89.6 years
Vulnerable: 949.1 years
i.e., approximately one order of magnitude difference in *Te* between Critically Endangered and Endangered and between Endangered and Vulnerable.

If generation time is longer than 3–4 years then t can be altered accordingly to determine the new threshold. For example, the Critically Endangered threshold for a species with an average generation time of 20 years (t = 60 years) would be 86.6 years.

A note of caution is needed before moving to the examples. Given the

simplicity of the model and the fact that *Te* is based on probability theory, *Te* should be interpreted as a relative risk of extinction rather than an absolute measure. Everything else being equal, a species with a *Te* of (say) 100 is more at risk than a species with a *Te* of 1000.

To illustrate the calculation of *Te* and the problems of estimating the parameter values we will consider a 23-year census of the green-winged orchid *Orchis morio* in Lincolnshire, England (Silvertown *et al.* 1994) and a 27-year census of the Bay checkerspot butterfly *Euphydryas editha* ssp. *bayensis* in California (Harrison *et al.* 1991). Whilst these are only local populations and therefore not necessarily representative of risk to the whole species for which the IUCN criteria and categories are designed, the methods and conclusions are of wider application. We therefore use local populations and samples of local populations as models of the dynamics of a species if it was reduced to that size and ask what would be its chances of survival and what data are needed to accurately assess that chance.

Time to extinction for *Orchis morio* and *Euphydryas editha*

An *Orchis morio* population in Bratoft Meadow, Lincolnshire, has been subjected to eight fertiliser treatments in a blocked factorial design with eight replicates (i.e., a total of 64 plots, details in Silvertown *et al.* 1994). Analysis of the effects of treatments on time to extinction will be dealt with elsewhere (Gillman & Dodd unpubl.). Effects of treatments on population density are dealt with by Silvertown *et al.* (1994). Here we concentrate on the total number of flowering plants in unfertilised plots (Figure 1), each of which is 1.83 x 9.14 m.

Using Eqns 3 and 4, the *Te* for each *Orchis morio* plot was calculated over the full 23-year time-span (Table 2).

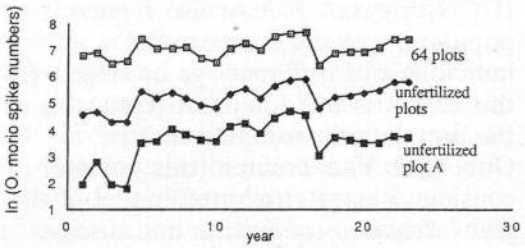

Figure 1. Change in numbers of *Orchis morio* flowering spikes in unfertilised plot A, all unfertilised treatment plots and all 64 plots.

Table 2. Estimates of variance in r (v_r), ln(maximum population size, k) and time to extinction, *Te* (from ln (k) and from ln ($N_{0)}$ = 4) for *Orchis morio* in eight unfertilised plots (A–H), plots A–H together and all 64 plots in experiment. v_r is calculated as variance of ln (N_{t+1}/N_t).

Plot	v_r	ln (k)	Te (k)	Te (ln(N_0) = 4)
A	0.398	4.718	55.9	54.6
B	0.485	4.394	39.8	39.5
C	0.667	4.060	24.7	24.7
D	0.366	4.060	45.0	45.0
E	0.752	3.738	18.6	18.5
F	0.284	4.564	73.3	72.2
G	0.483	1.609	5.4	– N_0>K
H	0.614	3.367	18.5	17.8
plots A-H	0.177	5.90	197	
all 64 plots	0.186	7.688	318	

Even though these are samples from the same population the eight plots differed by an order of magnitude in predicted *Te* (5.4 to 73.3 with N_0 = k). With N_0 = 4 there was a similar spread of values. Increasing the sample size to eight unfertilised and then all 64 plots increased the *Te* because of the larger k, although v_r was very similar between the 8 and 64 plots. To determine the effect of census duration we will concentrate on unfertilised plot A. This is reasonable as there was a high temporal correlation between plot A and the other seven unfertilised plots and between the unfertilised plots and the fertilised ones (correlation coefficients r = 0.890 and 0.894 respectively, Figure 1).

For unfertilised plot A, v_r, k and *Te* were calculated over durations of 3 to 23 years inclusive (with N_0 given as the population size at the last point in time).

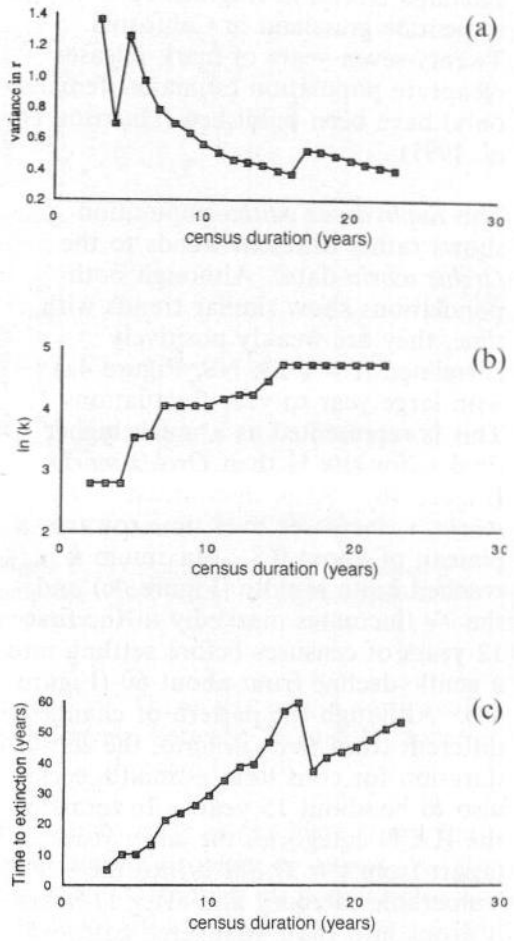

Figure 2. Change in (a) variance in r, v_r; (b) ln (k) and (c) time to extinction, *Te*, for *Orchis morio* in unfertilised plot A with census duration.

Thus at any year t we estimate the time to extinction from the current value of N based on information contained in that census duration. v_r declined exponentially with time towards an asymptote of about 0.4 (Figure 2a). k increased from less than 3 to close to 5 at which it remained after 15 years (Figure 2b) and *Te* increased almost linearly for the first 15 census years to a *Te* of 60 years, decreased, and then climbed back towards 60 (Figure 2c). This suggests that about 15 years of census data are needed in order to produce a reasonable estimate of v_r, k and, therefore, *Te* for *Orchis morio*.

Using the IUCN criterion of probability of extinction over the 23-year period, one subpopulation falls into the Critically Endangered category and seven into the Endangered category (Table 2). If the eight unfertilised plots or all 64 plots are considered, the population falls into the Vulnerable category. (Note that the criterion is one of several – the category applies if one or more criteria are met). For unfertilised plot A the critical threshold is passed after 6 years, after which the population remains Endangered (Figure 2c).

The issue of habitat fragmentation and metapopulation dynamics needs to be mentioned as this is a means by which local population dynamics can contribute to the dynamics of the species. For example, local populations of *Orchis morio* may be widely separated in space, as in Kent, England (Figure 3). For a metapopulation to remain extant, colonisations need to balance extinctions. The wide separation of local populations is not necessarily a problem if suitable habitat remains for colonisation. Unfortunately we know this is not the case for this species and for many others (Fuller 1987). Therefore we may need to view *Orchis morio* and many other species as a set of separate local populations with the time to extinction of the species equal to the time to extinction of the longest-persisting local

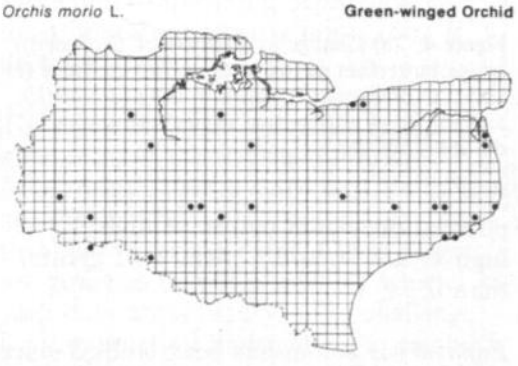

Figure 3. Distribution of *Orchis morio* in Kent (from Philp 1982 with kind permission of the author).

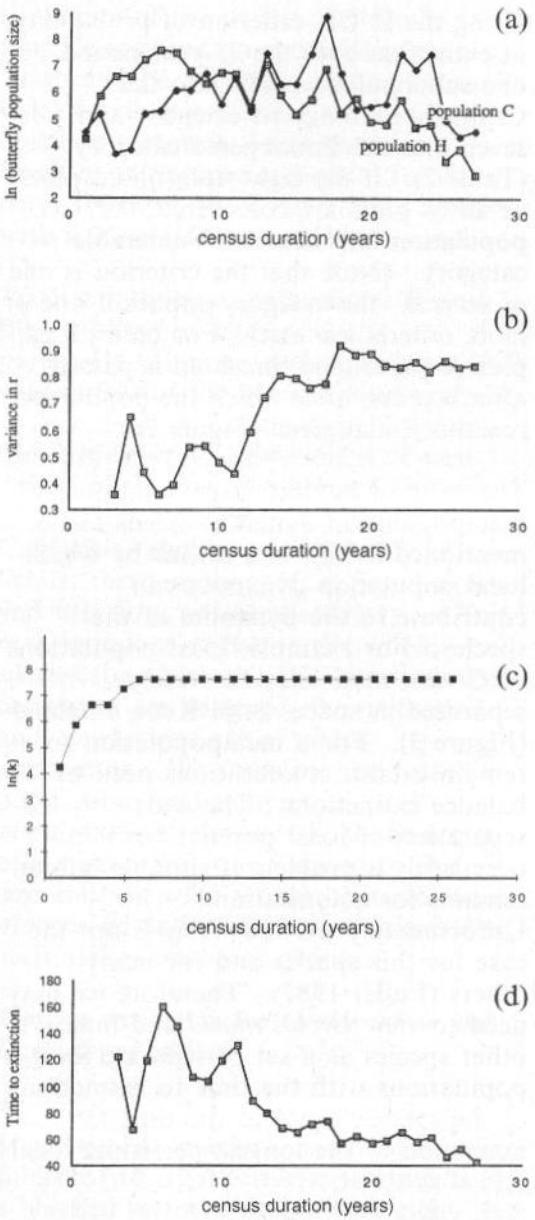

Figure 4. (a) Change in numbers of *Euphydryas editha* butterflies at two sites on Jasper Ridge (H and C), California. Change in (b) v_r, (c) ln (k) and (d) *Te* of *Euphydryas editha* with census duration for site H.

population which will have high N_0, high k, low v_r and a mean r of greater than 0.

Euphydryas editha has been studied since 1960 by Ehrlich's group at Stanford University. The species is oligophagous, feeding primarily on an annual plantain, *Plantago erecta*, in fragmented serpentine grassland in California. Twenty-seven years of mark-release-recapture population estimates (females only) have been published (Harrison *et al.* 1991).

The *Euphydryas editha* population shows rather different trends to the *Orchis morio* data. Although both populations show similar trends with time, they are weakly positively correlated (r = 0.23, NS, Figure 4a) with large year to year fluctuations. This is represented as a much higher final v_r for site H than *Orchis morio* (Figure 4b). Also, in contrast to *Orchis morio*, v_r increases over time towards a plateau of about 0.8. Maximum k is reached quite rapidly (Figure 4c) and the *Te* fluctuates markedly in the first 12 years of censuses before settling into a gentle decline from about 60 (Figure 4d). Although the pattern of change is different from *Orchis morio*, the census duration for consistent estimates seems also to be about 15 years. In terms of the IUCN categories the early years (apart from t = 3) put it into the Vulnerable category and after 11 years it drops into the Endangered category. This is the opposite to the *Orchis morio* example where short-term census data provided a less favourable view of its chances.

From a conservation perspective the importance we attach to 'small' depends on the sensitivity of *Te* to N_0. Plotting *Te* against N_0 for the two species (Figure 5) illustrates the problems of using single population size measures to estimate probability of extinction. For *Orchis morio* in unfertilised plot A we could use a single value of 20 as a yardstick for the change from Critically Endangered to Endangered (Figure 5a). But these data are confounded by census duration – the four shortest duration values are Critically Endangered. If this was the only extant population of the species under the IUCN criteria a value less than 50 would automatically put the species within the Critically Endangered

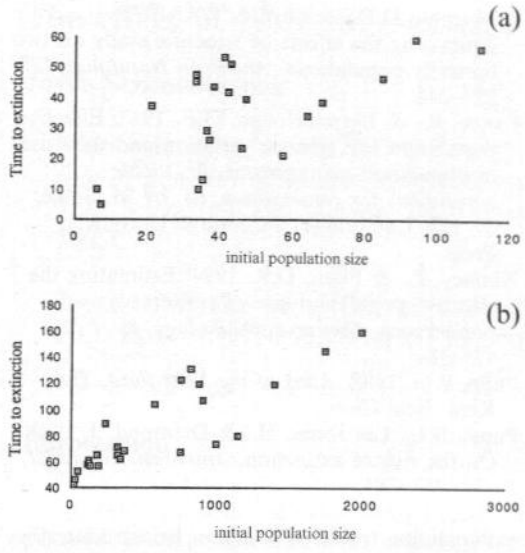

Figure 5. *Te* against N_0 for (a) *Orchis morio* unfertilised plot A and (b) *Euphydryas editha* population H.

category. There was only a very weak relationship between *Te* and N_0 once past the threshold of 20.

For *Euphydryas editha* there is not the confounding problem of census duration. Although all population sizes less than 370 are Endangered, a population size of 1150 is also Endangered. The population size of 370 is reasonably close to the 250 quoted as the single IUCN criterion for Endangered for one extant population. These data remind us that the notion of a magic number – the minimum viable population size in vogue at the end of the 1980s – must always be regarded with scepticism (Soulé 1987; Thomas 1990).

The analyses presented here point to the need to assess populations over reasonable periods of time and at appropriate spatial scales. It is interesting that the census data from the British butterfly monitoring scheme are now beginning to yield data on significant trends in change in population size with time, some 15 years after their inception (Pollard, Moss & Yates 1995). An important result of this work is that the same species can have significantly different trends in different places. Accurate estimation of trends with time are vital because negative *r* indicates a population heading deterministically towards extinction whilst positive *r* does not necessarily indicate a safe population but soon overrides the effects of variance in *r* on time to extinction (Figure 1 in Foley 1994).

Conclusion

The two studies highlighted in this paper provide a simple lesson in sampling for organisations concerned with conservation. If populations of short-lived species are to be accurately assessed for their chances of extinction then we may need censuses of the order of 15 or more years. Returning to the theme of the opening quote of Soulé (1987), the requirements for adequate data of the variables considered here are also relevant to the assessment of genetic criteria, such as effective population size, where estimation of generation time and fluctuations in numbers of adults are critical (Nunney & Elam 1994). For example, the susceptibility of a population to bottleneck effects can be estimated from a knowledge of the fluctuations in population size with time. The results of Ouborg & Van Treuren (this volume) suggest that inbreeding depression will increase the probability of extinction under certain conditions, although it seems that population dynamics are the driving force of local extinction – inbreeding depression is most important when a population is declining.

The efforts of a co-ordinated group of volunteers in the British Butterfly Monitoring Scheme show that, in Britain at least, a target of 15 years sampling is feasible for certain taxa. Clearly there are going to be other taxa for which such data are a luxury. The challenge for conservation biologists is to establish a set of sampling criteria which match the criteria laid down by IUCN and which address the linked dynamic and genetic attributes of populations. These

sampling criteria are likely to be nested in desirability from identification of a species through to repeated sampling of the abundance of a species over long periods of time at a number of locations.

Acknowledgements

We thank Mike Dodd for his help with analysis, Derek Wells and staff at the Lincolnshire Trust for Nature Conservation and others who established the *Orchis morio* experiment and collected the data (see Silvertown *et al.* 1994) and Heather Robertson (English Nature) for discussions of the *Orchis morio* experiment.

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The effect of inbreeding on seed germination

Joyce Schoondergang

Schoondergang, J.M.B. 1997. The effect of inbreeding on seed germination. *In*: *The role of genetics in conserving small populations*, ed. by T.E. Tew, T.J. Crawford, J.W. Spencer, D.P. Stevens, M.B. Usher & J. Warren, 163–164. Peterborough, JNCC.

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Introduction

Fragmentation and reduction in population size are likely to increase the probability of extinction of populations through demographic and environmental risks. Small populations are also expected to suffer from inbreeding depression and loss of genetic variation due to genetic drift. However, the exact impact of genetic erosion is still unclear.

The purpose of this project was to investigate the effects of genetic erosion in small scabious *Scabiosa columbaria*. In The Netherlands this is an endangered species which occurs in small as well as large populations. Here I report on the effects of inbreeding, and consider to what extent seed germination is affected by the level of inbreeding.

Methods

Seed collected from four natural populations of *S. columbaria* (population sizes: N = 35, 118, 5000 and 10000) was used to create lines with different degrees of inbreeding.

These lines were obtained by:
- outcrossing (inbreeding coefficient F = 0);
- full sib mating (F = 0.25);
- three generations of repeated selfing (F = 0.5, 0.75, 0.875).

For seed samples derived from these lines (n = 400) the following characters were measured:
- germination proportion: the fraction of seeds that germinated within 19 days;
- germination rate: the number of seeds germinated at a given day divided by the total number of seeds germinating.

Results

Figure 1 illustrates the effects of inbreeding on both germination proportion and germination rate for each of the four natural populations.

Conclusions

For three of the four populations, prolonged selfing (F > 0.5) had a negative effect on the proportion of seeds germinated. The Zalk population was an exception; here, the increase in germination for F > 0.5 might indicate purging of deleterious alleles. The degree of inbreeding had no consistent effect on the germination rate. For both germination rate and germination proportion, there was no clear-cut effect of population size. No relationship was found between the proportion of seeds germinated and the germination rate.

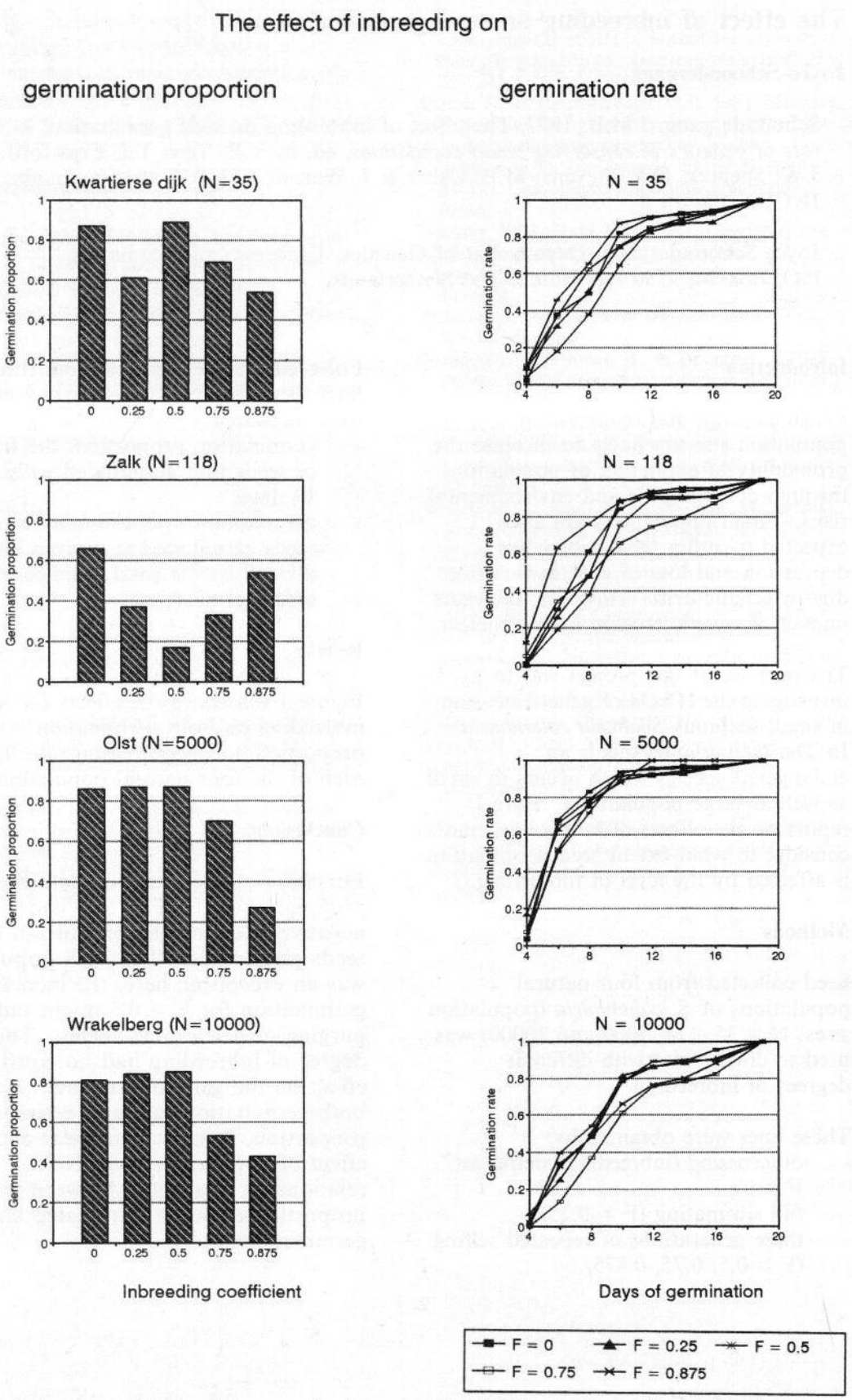

Figure 1. The effects of inbreeding on germination proportion (left hand column of graphs) and germination rate (right hand column of graphs) on four natural populations of *Scabiosa columbaria* with differing population sizes.

Pollen exchange by bumble bees in *Salvia pratensis*

Odilia Velterop and Manja M. Kwak

Velterop, O., & Kwak, M.M. 1997. Pollen exchange by bumble bees in *Salvia pratensis*. *In*: *The role of genetics in conserving small populations*, ed. by T.E. Tew, T.J. Crawford, J.W. Spencer, D.P. Stevens, M.B. Usher & J. Warren, 165–168. Peterborough, JNCC.

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Introduction

Recently many plant populations have become fragmented, often due to habitat destruction, and the resulting small, isolated populations face an increased risk of extinction due to demographic and environmental stochasticity. In addition, these populations will experience a loss of genetic variation and increased homozygosity due to genetic drift and inbreeding. These processes can reduce both the fitness of small populations (inbreeding depression) and their adaptability to a changing environment. Gene flow between remnant populations can alleviate the deleterious effects of small population size and isolation.

In many plant species with limited seed dispersal, gene flow occurs mainly by pollen, and this pollen flow often depends on pollinating insects. This paper details an investigation into gene flow via pollen in *Salvia pratensis*, a rare and declining plant species in The Netherlands, in which pollen flow is mediated by bumble bees. The bee's behaviour determines the pattern of pollen flow (and may consequently affect the degree of inbreeding) within the remaining small and isolated populations of *Salvia pratensis*. Bumble bee behaviour is known to be related to the size of the display of individual plants and this is expected to be especially important at the low plant densities typically found in fragmented populations.

By monitoring the foraging paths of individual bumble bees we addressed the question of how pollinator movement, and thus potential pollen exchange, is affected by plant size (here the number of open flowers) and plant position in the field.

Methods

In a Dutch population (Ruitenberg) of *Salvia pratensis* we marked all plants (n = 38) in an area of 110 m x 30 m, and we determined the number of open flowers per plant (Figure 1a). Almost all bumble bees (n = 65) foraging in the area were marked individually. On 22 June 1995 we observed the same transect 22 times and noted plant number, bumble bee number and time of the day. Bumble bee movements were reconstructed on the basis of resightings of individual bumble bees within 20 minutes, with most resightings occurring within 5 minutes.

Results

To analyse bumble bee movements over the area we assigned each plant to one of eight 'patches'. It is reasonable to assume that bumble bees are attracted by such patches, and do not differentiate between adjacent plants. We found all patches in the plot to be connected by bumble bee movements, although some flight paths were more frequent than others (Figure 1b). There were strong differences in behaviour between individual bumble bees (Figure 2).

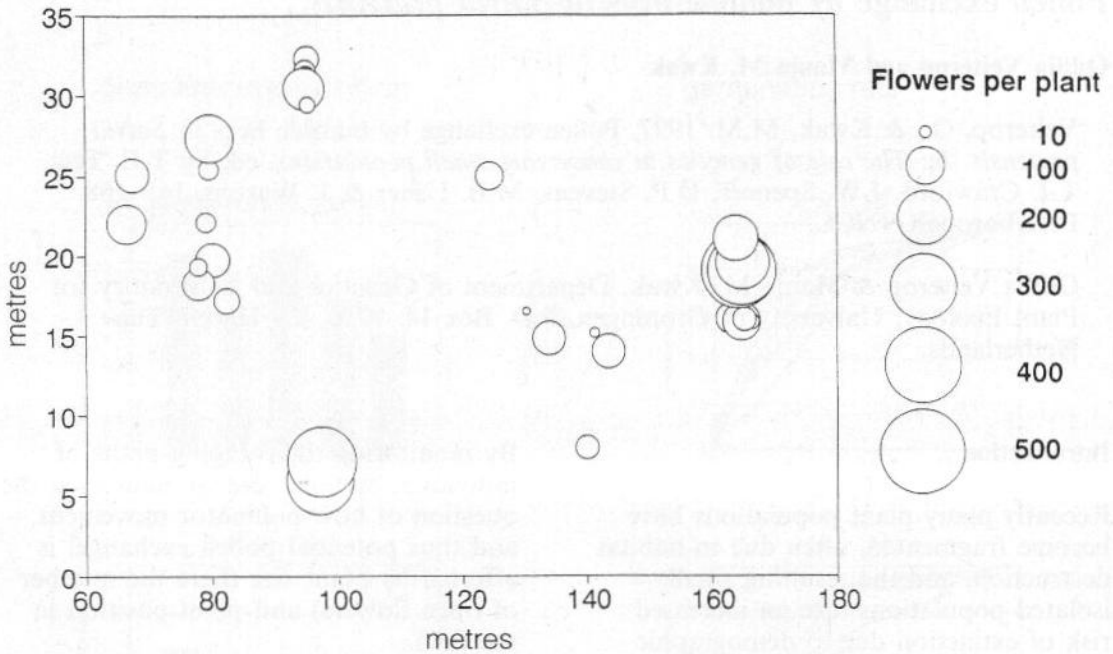

Figure 1a. Location of individual *Salvia pratensis* plants in the area. The size of the circles corresponds to the number of open flowers per plant.

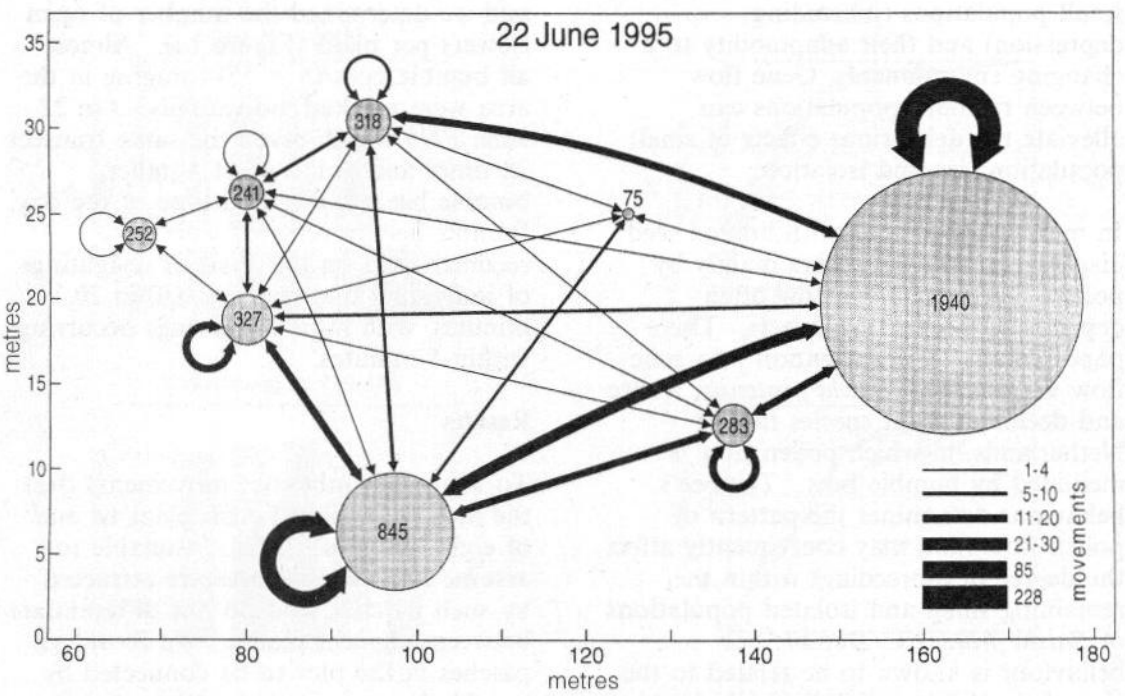

Figure 1b. Overall movements of bumble bees between patches of adjacent *Salvia pratensis* plants (n = 507).

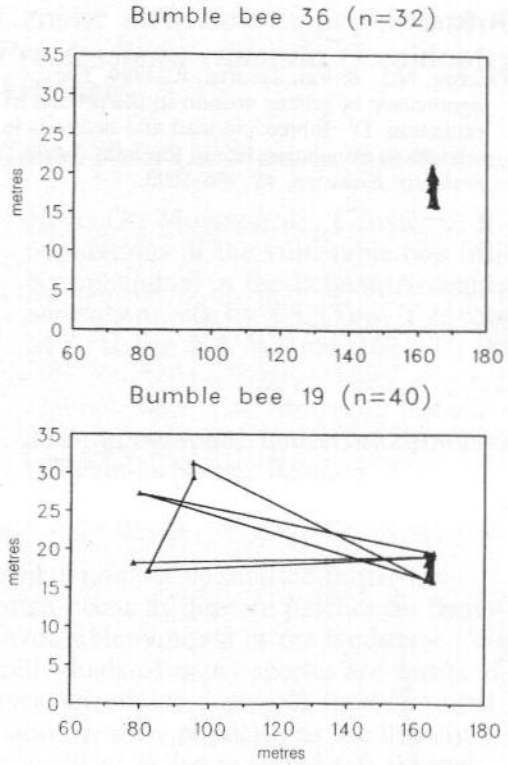

Figure 2. Two extreme examples of the foraging paths of individual bumble bees.

Some bumble bees had a restricted foraging area, whereas others visited nearly all plants in the plot. As a consequence, opportunities for pollen exchange can depend on the individual bumble bee visiting a plant.

As well as differences between bumble bees, individual plant sizes (number of open flowers per plant) can be important for visitation patterns. A negative relationship was found between the number of open flowers per plant and the percentage between-plant movements (i.e. pollen exchange; Figure 3). In other words, larger plants experience more geitonogamous visitation, that can cause relatively higher selfing rates. At the same time, plants with many flowers were connected with a larger number of neighbouring plants, thus having more diverse opportunities for pollen exchange than small plants (Figure 4).

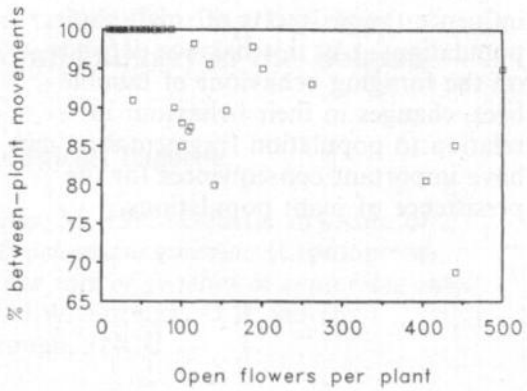

Figure 3. The relation between the number of open flowers per *Salvia pratensis* plant and the percentage between-plant movements of visiting bumble bees.

Discussion

Large plants have more connections to other plants, which may result in a genetically more diverse progeny. At the same time, they experience relatively more intra-plant movements, resulting in higher selfing rates. As Ouborg & Van Treuren (1994) have shown, increased selfing may lead to reduced progeny fitness due to inbreeding depression.

The balance between selfing (and the possible occurrence of inbreeding depression) and outcrossing (connections to other plants and gene flow) will

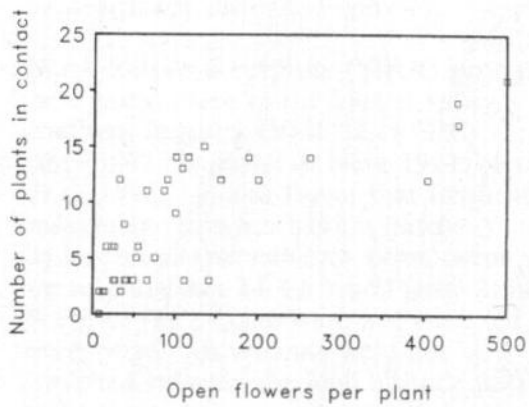

Figure 4. The relation between the number of open flowers per *Salvia pratensis* plant and its neighbourhood size (i.e. the number of plants connected to it by bumble bee flights).

influence the prospects of small plant populations. As this balance depends on the foraging behaviour of bumble bees, changes in their behaviour in relation to population fragmentation can have important consequences for the persistence of plant populations.

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Genetic structure of populations of the vulnerable bog fritillary *Proclossiana eunomia* (Lepidoptera, Nymphalidae) in the Belgian Ardennes

Gabriel Nève, Luc Mousson, Isabelle Convié and Michel Baguette

Nève, G., Mousson, L., Convié, I., & Baguette, M. 1997. Genetic structure of populations of the vulnerable bog fritillary *Proclossiana eunomia* (Lepidoptera, Nymphalidae) in the Belgian Ardennes. *In*: *The role of genetics in conserving small populations*, ed. by T.E. Tew, T.J. Crawford, J.W. Spencer, D.P. Stevens, M.B. Usher & J. Warren, 169–171. Peterborough, JNCC.

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Populations of specialised butterflies often occur as discrete patches on their favourable habitats in the landscape. As individuals of many species are rarely, if ever, encountered outside their favoured habitats, their populations are usually thought of as being closed (cf. Warren 1992). With this framework in mind, we started an investigation of the spatial structure of populations of the bog fritillary *Proclossiana eunomia*, a species occurring in wet hay meadows where bistort *Polygonum bistorta*, its only food-plant in middle Europe, grows. This species was chosen as a good example of a species with typically disjunct populations, because of its specific habitat requirements and its local abundance in suitable habitats, features which are typical of most vulnerable butterfly species in Belgium (Goffart, Baguette & De Bast 1992). Three complementary approaches were chosen to tackle the question of the causes and consequences of its spatial population structure : (1) genetic studies using allozyme electrophoresis allowed an assessment of the genetic relationships of populations; (2) Mark-Release-Recapture surveys were conducted to estimate the dispersal potential of this species; and (3) behaviour studies were carried out to infer the proximate factors of dispersal flights.

Genetic studies, using allozyme electrophoresis on cellulose acetate plates (Richardson, Baverstock & Adams 1986; Wynne, Loxdale & Brookes 1992), were conducted on the four locally polymorphic loci found out of the 10 loci polymorphic in Europe (adenylate kinase, E.C. number 2.7.4.3.; phosphoglucomutase, 2.7.5.1.; glucose-phosphate isomerase, 5.3.1.9.; 6-phosphogluconate dehydrogenase, 1.1.1.44). Within the Plateau des Tailles system (southern Belgium, 50·14'N 5·47'E), 286 individuals captured between 1991 and 1993 from seven samples (Figure 1) were scored for these enzymes. Genetic differentiation was assessed by the GENEPOP software (Raymond & Rousset 1995 a & b). Four different populations were then found to be genetically distinct (Figure 1).

Mark-Release-Recapture (MRR) surveys have taken place in the Lienne valley, southern Belgium (50·18°N, 5·49°E) since 1992 (Baguette & Nève 1994; Nève *et al.* 1996). It was found that frequent movements between habitat patches, across unsuitable habitats, often occur, up to a distance of 4.6 km (Figure 2). Some of the patches were not occupied every year. Movements were not observed between the high altitude peat bog area and the nearby valleys.

Behaviour and simulation studies suggest that mate density is a key factor causing dispersal behaviour out of habitat patches. Males tend to emigrate when

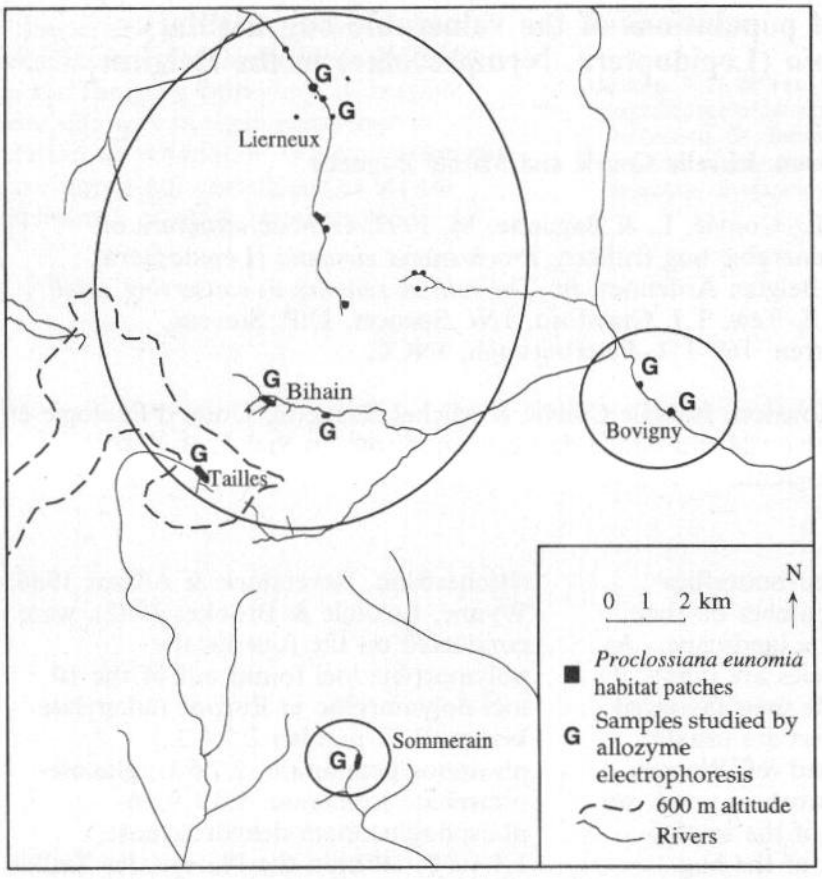

Figure 1. Populations of *P. eunomia* within the Plateau des Tailles system. Ellipses group patches which were shown to form single genetic units. The upper group was found to be a single unit, using both MRR and genetic techniques; the other two only by genetic methods.

there is a low density of females and females tend to emigrate when there is a high density of males (Baguette, Convié, Vansteenwegen & Nève, unpublished data).

This pattern of movements between patches leads to a metapopulation structure (Gilpin & Hanski 1991) at the landscape scale, which involves colonisation and extinction events. In hay meadows, mowing should not be carried out in all meadows simultaneously, but in a sequence of years to allow re-colonisation to occur. From both genetic and MRR data, the peat bog area does not seem to function as a source for regular recolonisation of nearby valleys, unlike the system of the checkerspot butterfly *Euphydryas editha* populations studied in Colorado by Harrison, Murphy & Ehrlich (1988). The metapopulation system of *P. eunomia* studied here is thus likely to function as a set of local populations with different probabilities of survival and recolonisation, according to the size and the history of the different patches, typical of what Harrison (1994) calls 'patchy population'. The long-term conservation of *P. eunomia* is therefore linked with the continued existence of networks of its habitat. In *Melitaea cinxia*, the fact that the network of suitable sites went below a critical threshold is probably the cause of its extinction in mainland Finland (Hanski, Kuussaari & Nieminen 1994).

A similar study on the cranberry fritillary *Boloria aquilonaris*, a nymphalid butterfly dependent on cranberry *Vaccinium oxycoccos* on peat bog areas, was launched in 1995. The greater isolation of its populations and its more stable habitats, compared with *P. eunomia*, suggested isolation factors to be stronger. An MRR survey carried out in 1995 also in the Plateau des Tailles area proved otherwise, as movements of up to

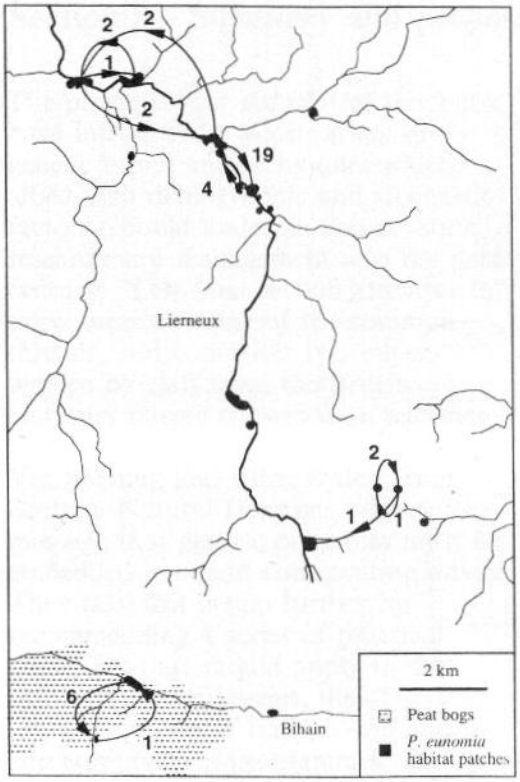

Figure 2. Movements of *P. eunomia* imagines observed between habitat patches in 1994. Numbers indicate the numbers observed between the patches. In 1993, when a higher density occured, movements were observed up to 4.6 km, but still no movements could be observed between the peat bog area and the nearby river systems.

10 km were recorded. The genetic structure of the *B. aquilonaris* populations will be studied both with allozyme and DNA techniques.

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Section 5 – Summary and proposals

The previous four sections of this book have introduced a wide variety of genetic topics and techniques which, allied with demographic and stochastic factors, should underpin conservation research and management into the next century. This final section attempts to draw together some of the common threads, and comprises two papers written by staff from the British statutory nature conservation agencies.

Vin Fleming and Chris Sydes, from Scottish Natural Heritage, endorse the message that genetic principles must be embedded in sound conservation advice. They take this a step further by recommending a series of practical guidelines that should apply to the prioritisation of species, the implementation of both *ex-situ* and *in-situ* conservation programmes, the provenance of source material, and the structure and size of populations and metapopulations. Consideration of guidelines such as these is critical for the long-term success of many recovery or action programmes.

Finally, David Stevens and Tim Blackstock, from the Countryside Council for Wales, consider the role of genetics in biodiversity conservation programmes in Britain. They make six proposals:

- the maintenance of genetic variation should be an explicit aim of biodiversity conservation;
- policies for site safeguard, species recovery, habitat restoration and *ex-situ* conservation should be based on sound genetic principles;
- species need to be prioritised for genetic research and conservation;
- genetic surveys of priority species should be commissioned;
- the extent to which genetic variation in priority species is represented within protected sites should be reviewed; and
- innovative research on the consequences of genetic impoverishment for population viability should be encouraged.

It is hoped that anyone reading this book will agree that their six proposals are a fair reflection of much of the content.

The science of conservation genetics is youthful and vigorous. Like all young sciences it sometimes seems to raise as many problems as it solves. We must not expect conservation genetics to answer all the problems – it is just one component of a multidisciplinary approach to biodiversity conservation. Nevertheless, conservation genetics is already providing answers and directions to nature conservationists at both strategic and practical levels. Not only must we take heed of the current results of genetic research, we must also look forward imaginatively to integrate this new science into future nature conservation policy and practice.

Genetics and rare plants: guidelines for recovery programmes

L.V. Fleming & C. Sydes

Fleming, L.V., & Sydes, C. 1997. Genetics and rare plants: guidelines for recovery programmes. *In*: *The role of genetics in conserving small populations*, ed. by T.E. Tew, T.J. Crawford, J.W. Spencer, D.P. Stevens, M.B. Usher & J. Warren, 175–192. Peterborough, JNCC.

Conservation bodies are increasingly involved in the active management of threatened plants in the UK. Incorporation of genetic principles into such work at an early stage is both possible and desirable. We consider roles for genetics research and screening in selecting species and populations at risk and for sampling populations in order to adequately represent the genetic element of biodiversity. The lessons derived from recent research are examined in order to suggest guidelines to apply to the role of *ex-situ* cultivation and in choosing methods for *in-situ* restoration. Genetic research can also be applied to choosing the sources for original material and for making decisions about the structure of the final populations such as the development of functional metapopulations. Properly designed and recorded recovery attempts are a useful tool for gathering empirical evidence on the significance of genetic effects on population fitness.

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Introduction

Traditionally, site and species protection have been the key elements of any strategy for nature conservation (Nature Conservancy Council 1984). Just over 11% of Scotland's land surface (8% of the UK), for example, is designated as Sites of Special Scientific Interest. These give a degree of protection against adverse changes in land use. Equally, some wild plants have been given special legal protection since the Wild Creatures and Wild Plants Act 1975, now superseded by the Wildlife & Countryside Act 1981. Over 108 British vascular plants are now protected against intentional uprooting, collecting and damage. Yet, because these mechanisms do not always mitigate the causal factors of extinction, populations of wild plants have continued both to decline and to become more isolated in increasingly fragmented landscapes.

Direct management of populations of rare species is becoming increasingly important as a means to maintain or restore species in the face of modern land use (Maunder 1992). Whilst such 'recovery' efforts have long been used for animal conservation, they are in their infancy for plant conservation in Britain. Priority for positive action has been given to species considered to be under the greatest threat with the degree of threat being largely determined by demographic and distribution data. Other factors, such as recovery potential, legislative priority and resource implications may also be pertinent (Whitten 1990). Rarely are genetic factors considered, though the inclusion of taxonomic distinctiveness as a selection criterion in some programmes assesses at least one measure of genetic diversity (Molloy & Davis 1992).

Typically, conservation efforts for plants have used techniques such as *ex-situ* cultivation, translocations and habitat management. In relatively few cases, however, have the results of genetic research been used to inform and guide the development of translocation guidelines (e.g. Birkinshaw 1991;

Gordon 1994) or recovery attempts (e.g. DeMauro 1994; Pavlik, Nickrent & Howald 1993; Jackson 1995). Yet the diversity and movement of genes within created or existing populations may have profound implications for their long-term persistence (Lande 1995). Equally, genetic diversity with its patterns of local adaptation and evolutionary lineage and potential is a significant element of overall biodiversity and worthy of conservation in its own right (Kay & John 1994; Fenster & Dudash 1994; Stevens & Blackstock this volume). However, Schemske *et al.* (1994) and Widen (1993) have suggested that demographic factors are likely to be the most significant consideration in plant population management and extinction risk assessment. Even so, it is both feasible and desirable to incorporate genetic principles to such work at an early stage. Not to do so, in the face of an increasing body of theoretical and empirical evidence on the subject (e.g. Falk & Holsinger 1991; Fenster & Dudash 1994), might be considered negligent on the part of conservation managers.

In this paper, we attempt to formulate a set of simple guidelines that inform conservation managers how genetic principles can be applied at different stages in the recovery of wild plant populations. They are meant to complement, not supersede, guidelines on translocations already in existence (e.g. Birkinshaw 1991; Species Survival Commission 1992) and management principles based on demography.

Selecting candidate species

Candidates for recovery are generally selected on a variety of grounds such as threat, rarity and observed decline in abundance. In particular, small isolated populations are known to be at greatest risk from demographic and environmental stochasticity (Belovsky *et al.* 1994; Schemske *et al.* 1994). Such populations are also at greatest risk from genetic processes such as drift, founder effects or inbreeding depression (Barrett & Kohn 1991; Nunney & Campbell 1993; Heschel & Paige 1995). Loss of genetic variability may eventually result in populations with reduced fitness and which may be less likely to respond to changing environments. Yet most populations of rare plants are small. Of a sample of 351 quantified populations of 29 Red Data Book plant species in Scotland, over 75% have no more than 100 individuals and less than 5% exceed 1000 individuals (Figure 1). At the extreme, some of these 'populations' may consist of a single genet. We have no reason to believe that this sample is atypical of the 1200 or so rare plant populations occurring in Scotland. If anything, this sample contains some of those species known to have unusually large populations.

Few rare plant populations, then, will exceed the 500 individuals suggested as the minimum needed to maintain genetic variation (Franklin 1980; Soulé 1980). Hardly any exceed the revised upper estimates of effective population size (>5000) suggested by Lande (1995). Given that the effective population size (N_e) for genetic processes is likely to be significantly smaller than the total population size (Nunney & Campbell 1993), the data suggest that many Scottish rare plant populations may be at risk from the consequences of the genetic processes that appear to take place in small populations. It is important to recognise though that not all small populations may be genetically depleted or subject to, say, inbreeding depression (Widen 1993; Widen & Andersson 1993; Hauser & Loeschcke 1994). Continued inbreeding may result in the purging of deleterious alleles from a small population.

Many populations of rare plant species may then be considered as high priority candidates for recovery on both demographic and genetic grounds. Given that resources are likely to be limited, can we develop criteria that may

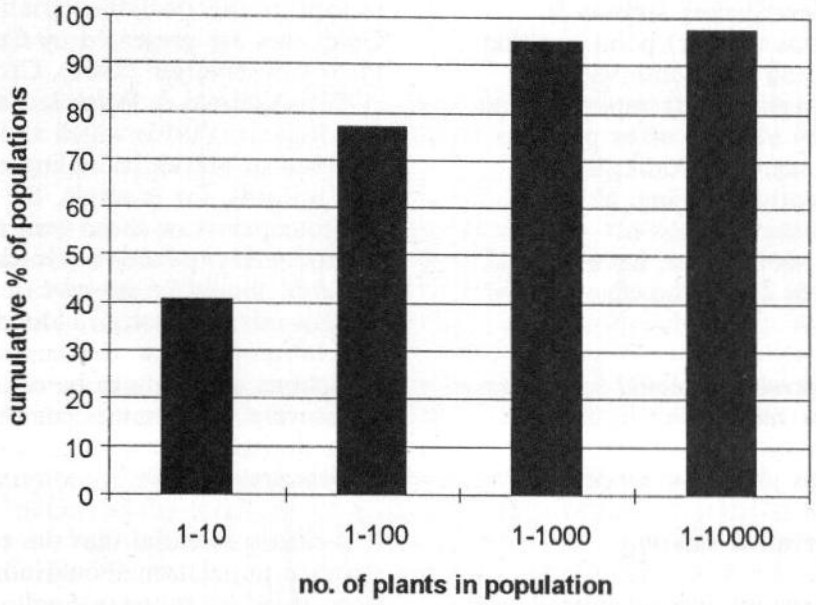

Figure1. Frequency of population size in a sample of 351 threatened plant populations in Scotland.

assist in defining priorities for initial research and genetic screening? Rare species are likely to be priorities for genetic screening where evidence or observations indicate the following:
(a) that populations consist of one or a few clones spreading vegetatively;
(b) where populations show signs of breeding failure;
(c) where populations are known to have recovered naturally, or been founded, from very low numbers;
(d) where metapopulations were formerly large but have suffered a dramatic loss of individuals and/or populations resulting in increasing isolation of survivors;
(e) where populations are very isolated from their nearest neighbours (and are suspected or known to be outcrossing species);
(f) relic non-recruiting but long-lived species.

Some populations or recently-arisen species may lack any effective variation, for whatever reason, and may not be subject to any obvious additional risk arising from that. In addition, not all nationally or internationally rare plant populations are small and some may be both large and dynamic within their niche or geographical range regardless of their genetic constitution. For example, the Scottish endemic, *Primula scotica*, has a relatively restricted world distribution but has 160 colonies with a total population estimated at 500,000 individuals. *Carex chordorrhiza* has hundreds of thousands of ramets although it is restricted to two locations in the UK (Cowie & Sydes 1995). Nevertheless, research on these species has been encouraged and supported by SNH (Glover & Abbott 1995; Ennos *et al.* this volume; Ennos *et al.* in press). *C. chordorrhiza* appears to consist of large spreading clones and at one of its sites has apparently expanded considerably. Long-term monitoring of *P. scotica* demonstrated breeding failures and it has been suggested that this reflected genetic differences between individuals (Bullard *et al.* 1987). Indications of concern about the genetics of threatened species may be derived from detailed census monitoring, from survey of populations and from autecological research and even the casual observations of experienced

botanists. Nevertheless Stevens & Blackstock (this volume) point out that the identification and conservation of genetic biodiversity is an important end in itself. They indicate other priorities for genetic research including species that are currently declining, have population concentrations of international significance, have disjunct distributions or are at the edge of their range.

Guideline: screening should be applied as a priority to species that are suspected to be at risk from the consequences of genetic processes.

Ex-situ conservation measures

Ex-situ conservation measures have an important role in the conservation of rare plants. First, they are an important resource for fundamental research, education and publicity. Second, they act as a safeguard against the extirpation of species or populations in the wild. They can also be used to capture existing genetic variation in rapidly declining species before such variation is no longer available to us. Third, they are important sources of plant propagules to return to the wild as part of any future co-ordinated recovery attempts.

Ex-situ conservation poses many risks to the capture or maintenance of genetic variation without loss or change (Birkinshaw 1991; Botanic Gardens Conservation International 1992; Gordon 1994; Kay & John 1994; Lesica & Allendorf 1992; Reinartz 1995). Methods of *ex-situ* conservation include maintaining (or breeding) living plants in cultivation or *in vitro* (Fay 1994; Rubluo *et al.* 1993), or maintaining seeds or spores in cold storage (Botanic Gardens Conservation International 1992).

For all these methods it is vital that an adequate sample of *in-situ* genetic variation is taken. The smaller the population the greater the proportion of the individuals that should be sampled to capture the available variation. Guidelines are presented by Center for Plant Conservation (1991), Crossa *et al.* (1993), Akeroyd & Wyse Jackson (1995) and Reinartz (1995) which should be followed to prevent sampling error or bias towards, for example, the most vigorous plants or those with most prolific seed production (Gordon 1994). Material should be stored carefully to prevent mixing between individual or population samples. Token, *ad hoc* collections are likely to be of little value in recovery programmes compared to those derived from a planned sampling programme.

It is clearly essential that the survival of a source population should not be jeopardised by propagule collection (Reinartz 1995). At the very least the total resource being exploited should be quantified in advance to assess the proportion being sampled. Seed banking may need additional exploitation of native populations because repeated sampling of seed is required to make up for both the loss of viability in storage and for seed used in the tests of viability.

Guideline: all sampling of genetic material from the wild should be part of a planned sampling programme with measures taken to avoid unconscious bias.

There are further risks from *ex-situ* cultivation. Briefly, if outbreeding sexual reproduction occurs then accidental loss of traits may occur due to genetic drift and to adaptive selection within the 'garden' environment (Botanic Gardens Conservation International 1992; Kay & John 1994; Pigott 1988; Reinartz 1995). After a few generations of selection within a garden, the progeny may have low survivorship in native locations compared to plants exposed to stress in the wild (Huenneke 1991; Lesica & Allendorf 1992). For example, Pavlik, Nickrent & Howald (1993) found that propagules derived from a cultivated population of *Amsinckia*

Genetic screening

It may be useful to assess the existing pattern of genetic variation in plant populations being considered for use in species recovery programmes. There are two main methods used to do this at present.

The first method is to assess markers in the genetic material of the plants by isoenzyme electrophoresis or by analysis of DNA polymorphism. These markers are thought to be selectively neutral but may still reveal the presence and scale of variation and relationships between individuals and populations. The equipment and expertise required, particularly for work on DNA, is expensive but organisations where it occurs may be interested in applying their skills to a practical problem. The methods may not be usable where biochemical complications, such as secondary compounds, prevent suitable extracts being obtained. It is also possible that the limited number of isoenzyme markers available for analysis, which represent a minute fraction of the total genetic material of any species, may reveal little or no variation even though it occurs elsewhere on the genome. Living tissue is required from individuals in the target populations. This is not always available from a much reduced population but material can be obtained by growing new individuals from wild seed.

The second major method is to assess quantitative characters expressed in the phenotype of individuals in population or family trials. This method has the potential advantage that it can tell us about the variation in characters that could be of direct ecological importance. An example of this approach for *Primula scotica* is described by Ennos *et al.* (this volume).

The important question of whether inbreeding and outbreeding depression will occur in target populations can be quite simply assessed by carrying out crosses between populations and measuring the performance of the seedlings. However, great variation has been found between the results of tests carried out *in-situ* and *ex-situ.* A garden environment presumably allows affected progeny to perform better than in harsher natural conditions.

The population size and breeding system of the target organism may itself provide sufficient indication of the likelihood and nature of genetic problems within the species. For example, obligate selfing species are less likely to suffer inbreeding depression when found in small populations. In comparison, obligate outbreeders in small, isolated populations are most likely to have suffered or be suffering rapid reductions in variation. Less conspicuously, the self-incompatibility genes used by some species to prevent self-fertilisation can prevent matings between two plants of the same mating type and may prevent or depress seed production in small populations (Demauro 1994). Seed-ovule ratios can also be determined and poorly-performing populations examined further, for instance by identifying the site of pollen tube inhibition after controlled crosses.

grandiflora had lower genetic variation than those derived from a wild population despite a common origin, though this did not correlate with any measured loss in fitness. Moreover, living collections might conceivably hybridise with other material of different provenance or with garden strains or hybrids of the same species (Birkinshaw 1991). Kay & John (1994) suggested that genotypes which may be favoured in the garden environment include those

with precocious growth and flowering, with reduced seed dormancy and that produce abundant seed. Other problems may arise due simply to human error or poor management, such as mixing accessions or wrong labelling (Botanic Gardens Conservation International 1992). Indeed, Barrett & Kohn (1991) suggest that "*A major challenge of* ex-situ *conservation will be to ensure that sexually propagated samples of rare plants do not become museum specimens incapable of surviving under natural conditions*".

Significant and visible problems in the maintenance of *ex-situ* animal populations have meant that zoological gardens now apply rigid controls to maximise outbreeding (Lacy 1994) while controlled inbreeding is also used as a method to express and eliminate deleterious alleles. Though relatively expensive they would prevent the changes in genetic variation which are likely to occur in outbreeding species maintained in small numbers in cultivation. The techniques of controlled plant breeding are regularly used in the development of new varieties in agriculture and horticulture and their application to populations of threatened plants which have to be maintained *ex-situ* might be appropriate.

Unlike most animals, plants are capable of vegetative regeneration and many species can be maintained indefinitely in cultivation without suffering any genetic change (apart from that derived from rare somatic mutations). Rigorously preventing sexual reproduction could largely prevent undesirable genetic change but has only been occasionally applied (Pigott 1988). As an additional safeguard, material derived from different populations or genetic provenance should not be grown simultaneously or in close proximity unless measures are taken to prevent any possibility of crossing (Birkinshaw 1991; Kay & John 1994). Outbreeding annuals present some difficulty in this respect and seed or spore banks may offer a more realistic alternative for them.

Guideline: perennial species in ex-situ *collections must be maintained without sexual regeneration unless they are apomictic or otherwise rigorously selfing species. Outcrossing annuals are best maintained in seed banks until required for cultivation.*

However, maintaining clones is also not without its hazards. Firstly, clones may also be subjected to differential survival, especially if not spatially separated. Clones less suited to the garden environment may be gradually eliminated or swamped by more successful clones. Secondly, unless the target species has a small total population size, it may be expensive and difficult to sample the full range of genets available in surviving populations. Fortunately, isozyme screening is likely to be an effective way of identifying genets within most populations. Overall it is clear that a system designed to maintain genetic diversity as well as the mere appearance of a threatened species *ex-situ* is more costly than generally realised (Pigott 1988).

Guideline: clones set up for ex-situ *conservation must be separately maintained so that genets are not eliminated by competition.*

If, as is likely, species or populations chosen for recovery have reached a low ebb, material for restoration is likely to be limited. Bulking up is a normal horticultural technique and is likely to be necessary (Birkinshaw 1991). Here the possibility of rapid genetic selection is particularly acute. It can be eliminated by using vegetative methods of regeneration alone, but this may limit the variation in outplanted material (Gordon 1994). Adaptation is likely to occur to the soils, seed trays, watering regime and garden environment. Such insidious selection can only be limited by reducing the number of *ex-situ*

sexual generations to an absolute minimum. Ideally, plant material being produced for return to the wild should be derived directly from wild populations.

Guideline: material being grown for return to the wild should be derived directly from wild propagules wherever possible. If material needs to be bulked up horticulturally then the number of sexual generations in cultivation should be strictly limited.

Where a surplus of material is produced by sexual reproduction, the plants used for recovery must be randomly selected and not biased, as is the horticultural inclination, towards the larger and more healthy as these may not necessarily be so suited to the wild (Birkinshaw 1991; Reinartz 1995). They may not also represent the full range of genotypes present. Without strictly random sampling methods, unconscious selection is liable to occur at many selective stages such as at pricking out or seed collection. An incidental risk is that material may be subject to new pests, diseases or competitors which the wild populations might not experience (Gordon 1994; Kay & John 1994) again adding to selection pressures not experienced in the wild. Reinartz (1995) further suggested that horticultural practices within the nursery should be varied and that plants should be planted in blocks across a range of garden micro-habitats.

Guideline: seed or young plants should be selected by a rigorous random method at all stages in the process.

In-situ restoration

The ultimate aim of most recovery attempts is to restore viability to threatened populations in their native localities. Methods to achieve this range from habitat management to direct manipulation of populations by translocations. Both techniques and their implications are considered here.

Habitat management

This technique involves least intervention in normal population processes and it should, therefore, be considered as a first option in any recovery attempt. Yet genetic effects may still be encountered and should be considered in planning. For example, site management may restore favourable conditions enabling a population to expand naturally into suitable niches. Seedling establishment will then be subject to normal selection pressures largely free of anthropogenic bias. However, there may well be founder effects if the original population had been reduced to a few, perhaps genetically attenuated, individuals acting as the source of new recruits. Not all populations will be at risk and some species may be adapted to frequent cycles of bottlenecks and subsequent recovery without any significant loss of diversity (Coates 1992). If screening indicates risks from genetic processes then habitat management may still need to be supplemented by direct population management. Site management cannot affect the degree of isolation of a population unless it also removes barriers to gene flow.

Guideline: where feasible, habitat management should be used as a first option in the recovery of threatened plant populations (though attention should be paid to the number and genetic variability of source and regenerating plants).

Habitat manipulation may also be used to exploit the recovery of individuals from *in-situ* seed or spore banks (Jefferson & Usher 1987; Maunder 1992; Page *et al.* 1992). Such recruitment may enable the restoration of genotypes, and thus heterozygosity, lost from the contemporary population. Such an approach is not likely to be available for all species but spore or seed banks have been detected for a variety of ferns (Dyer 1994; Dyer & Lindsay 1992) and flowering plants (Jefferson & Usher

1987). They have been successfully exploited to restore species thought to have been locally extirpated, such as starfruit *Damasonium alisma* in southern England (Marren & Rich 1993). Ecological research on seed banks has been considerable (Thompson 1992) but the conservation and genetic implications have not been fully exploited. Experiments need to be directed at means of detecting and stimulating regeneration from seed banks of threatened species. Genetic screening could monitor changes to the genetic structure of a population as a result.

Guideline: attempts should be made to exploit in-situ *seed banks to increase numbers of individuals and genetic variation.*

Population manipulation

Direct manipulation of populations is most typically undertaken by the re-stocking, re-establishment or creation of new populations with plant material derived from elsewhere. Fenster & Dudash (1994) suggested that genetic issues are more pertinent to the restoration or creation of populations than to the conservation of existing ones. Direct intervention has the greatest potential effect upon the genetic structure of populations and also carries some of the greatest risks. Depending upon the method used or source of introduced plant material, manipulation can be used to counter inbreeding depression or founder effects but may also destroy local adaptation and co-adapted gene complexes so causing outbreeding depression (Barrett & Kohn 1991; Fenster & Dudash 1994). It may also damage that part of the scientific value of populations related to their genetic lineage and history (Kay & John 1994; Stevens & Blackstock this volume). It should, therefore, be approached with a considerable degree of caution and a full understanding of the demography, genetics and breeding system of the target plant.

Three methods are potentially available to establish plants in the wild, namely, (i) direct planting of cultivated material; (ii) direct sowing of seed; (iii) direct transfer of material from wild populations. Each of these are discussed in turn.

(i) *Direct planting* This is the approach most commonly used in recovery attempts. Typically, material cultivated in botanic gardens is planted out at field locations after suitable hardening-off (Maunder 1992). Such plantings are usually given considerable aftercare by watering, weeding or protection from grazing until they are fully established (e.g. Birkinshaw 1991; DeMauro 1994; Pigott 1988). The care they receive is a function of the resources that have already been committed to their prior cultivation and the desire for the project to succeed. Yet this approach may impose further artificial selection on transplants. For some species, transplanting is the only option available to conservation managers but the extent to which natural selection pressures are over-ridden should be kept to a minimum. Managers must accordingly be prepared to consider accepting greater losses of plants in such programmes and to incur the greater cost this entails. Where material available from a threatened population is necessarily very limited this may not be appropriate.

Guideline: translocations using cultivated material should keep any aftercare to the minimum.

(ii) *Direct seeding of field sites.* This has significant advantages because establishment should not be influenced by anthropogenic selection pressures. Individuals are only likely to survive in suitable niches and selection is likely to be near-natural. Establishment costs are also reduced because seed is relatively easy to handle (Birkinshaw 1991) and because aftercare can be minimal. We may need to carefully select the areas used for sowing to make best use of scarce seed resources. In some cases, it

may be necessary to create open areas likely to be most suitable for regeneration. If seed has to be derived from garden stock then, at least, an element of natural selection will be imposed upon its establishment.

Seeds may also be better than clonal (vegetative) material for establishing plants in novel environments (Huenneke 1991). Indeed, this method has been tested successfully with *Amsinckia grandiflora* (Pavlik, Nickrent & Howald 1993), with survival rates exceeding those reported for some nursery transplants (DeMauro 1994). It has also been proposed for other recovery programmes (Bowles *et al.* 1993). Plants established from seed are likely to be able to exploit crevices and other niches in a way that plants grown in containers may not (Birkinshaw 1991). It may, therefore, be a useful method for establishing plants in 'difficult' sites. The incidental risk of importing novel pests and diseases from the garden may also be avoided.

There are significant drawbacks to this method because it needs a large supply of seed which may not be available in the wild population and, if it is, collection may be time-consuming since it needs to be subject to rigorous sampling practice (Reinartz 1995). The balance of genotypes needs to be equally represented in any sowings and should not be biased by containing larger amounts of seed derived from the more prolific seed producers. The approach might be considered to be inefficient use of a scarce resource (Maunder 1992) and may be subject to significant losses, especially if sown into established vegetation without taking steps to reduce competition (e.g. Pavlik, Nickrent & Howald 1993). However, many rare plants occur in unstable environments in which new niches become available naturally and selective sowing can allow the plant to exploit these.

This method is unlikely to be applied to species at the very rarest end of the spectrum, species with minute seed or strongly mycotrophic species (orchids for example) but is potentially realistic for other prolific seeders. We have estimated the mean annual seed production per population for three threatened species in Scotland and found it to be 3000 seeds (*Phyllodoce caerulea*), 50,000 seeds (*Carex chordhorrhiza*) and 300,000 seeds (*Schoenus ferrugineus*). It seems unlikely that, in any one year, cropping a few thousand seeds of the two more productive species would harm the natural regeneration of these populations. Establishing new populations by direct seeding is likely to be slow to produce demonstrable results, a problem where resources are time-constrained or where there is pressure for early achievements. By contrast, it may allow the expression of individuals that are slow to germinate and which would be discarded in garden cultivation. It may be used as a method to supplement, and compare with, direct planting. Direct seeding should not, however, be used simply as a means of using unwanted seed.

Guideline: trial establishments using seed should be attempted where it is feasible to do so and may be used to supplement other translocation methods.

(iii) *Direct transfer of wild material.* This third option, which appears to be little used, is direct transfer of material (other than seed) from wild populations. This has potentially a direct and damaging impact on donor populations if individuals are to be uprooted, but may be less so if cuttings or other propagules, such as bulbils or corms, are used. This has some advantages in that material will be adapted to wild conditions and can be screened beforehand so that its genetic constitution is known. This method might be considered especially suitable where transfer of propagules or clones between populations is required to enable outcrossing between genetically different individuals.

Provenance of source material

The most important factor in relation to the genetic management of populations using any of the methods above is the source and genetic composition of translocated material. Traditionally, the conservation agencies have adopted the precautionary principle of advocating the use of local genotypes in plant translocations whether for use in small threatened populations or in creating new native woodlands. This approach will tend to maintain any local adaptation and co-adapted gene complexes, and preserve the genetic history and integrity of populations. It assumes that the greatest threat to the survival of small isolated populations subject to conservation management is that outbreeding depression will take place with less-fit individuals resulting from the introduction of new heterozygosity.

However, if a population is in decline because it is subject to inbreeding depression, or if variation is reduced such that only sexually incompatible individuals remain (DeMauro 1994), adopting this precautionary approach will only compound these problems. Whether inbreeding or outbreeding depression is a dominant factor, if either, can only be discovered by genetic screening and studies of breeding systems.

In the absence of evidence of these effects, the precautionary approach should be maintained although this may result in continued decline if inbreeding depression is a significant problem. Direct re-stocking of an existing population, if a priority on demographic grounds, should, therefore, only use genotypes selected from that population unless there is evidence to the contrary (Gordon 1994; Reinartz 1995).

Guideline: re-stocking of existing populations should use material derived from that population unless there is reason to suspect inbreeding depression or similar genetic-related problems.

If a small population is surviving because of successful adaptation to its local environment it might also be at considerable risk if a new and more variable population is established within easy pollen (or conceivably seed) transfer distance (Ellstrand 1992). The degree of risk depends on the circumstances. If co-adapted gene complexes occur then the population is most likely to be threatened if it is small, strongly outbreeding, if the number of niches for regeneration each year are very limited and if selection occurs strongly but only at intervals of years. Problems may be especially acute if the new population is larger than the native one with the risk that foreign pollen will 'swamp' the original population (Ellstrand 1992). Under these circumstances if distant pollen with less adaptive genes enters the population, regeneration will produce offspring with a range of fitness depending on the survival or break up of the complexes. Normally the natural selection that leads to the set-up of the co-adapted genes would be expected to operate again to remove the less-fit offspring. However, if selection takes place sporadically or at long intervals or the number of regeneration niches are few, then the population might be left with too few fit individuals to survive the exacting period. Reinartz (1995) accordingly suggested that, to reduce the likelihood of outbreeding depression occurring by gene flow, new populations should only be created at least 1 km from a native population.

Guideline: when creating new populations within regular seed or pollen transfer distance to an existing population, the presumption is to use material derived from the native population.

There are, however, an increasing number of field and glasshouse studies demonstrating the deleterious effects of inbreeding or breeding incompatibility

(e.g. Rathcke & Real 1993; Snow & Spira 1993; Johnston 1992; DeMauro 1994; Ouborg & Van Treuren 1994; Heschel & Paige 1995). These effects may be associated with population size, on some occasions being greater in small populations (Heschel & Paige 1995). Inbreeding depression and associated failure in breeding success may be particularly severe in outbreeding species (Frankham 1995) but can be significant even in highly selfing populations (Agren & Schemske 1993). If we have reason to believe that the detrimental effects of inbreeding depression or similar effects override the advantages of local adaptation or co-adapted gene complexes, then the restoration of heterozygosity to an existing population is a priority even if this damages local adaptation (Barrett & Kohn 1991; Birkinshaw 1991).

Guideline: restoring heterozygosity to an existing population is a priority where inbreeding depression is demonstrated.

In this case how should new genotypes be selected to mix in with native genotypes? The more distant a population the more likely it is to differ genetically and the greater the chance of different ecotypes being involved (e.g. Walters, Decker-Walters & Gordon 1994). Ideally, therefore, alternative sources of variation should be derived from geographically close populations (Birkinshaw 1991). This will not always be feasible as some of our rarest plants are now many tens of kilometres from their nearest neighbour.

Guideline: additional variation for re-stocking existing populations should be first sought from geographically close sources where possible.

A different approach may be appropriate when creating new populations or restoring lost, now isolated, colonies. For example, Barrett & Kohn (1991) suggested that wide crosses may provide a significant advantage for the exploitation of new sites and novel environments. In some cases, this may be the only option available as, for example, in the restoration of populations of Pitcher's thistle *Cirsium pitcheri* in North America (Bowles *et al.* 1993). There is some evidence that distant crosses between populations can increase fitness in populations whether they are inbred or not (Hauser & Loeschcke 1994; Heschel & Paige 1995). Where regeneration is vigorous, foreign genes which are not suited to their new locality should be rapidly selected out and the new population become closely suited to the site. This approach can be tested experimentally using populations of pure local or distant origin and mixtures of the two (Barrett & Kohn 1991; Maunder 1992; Mistretta 1994). Crossed progeny could be produced within the nursery (Reinartz 1995), given great care to prevent the other problems described earlier. The progeny and sources should be genetically screened and monitored for performance.

Guideline: new populations, established beyond the limit of regular pollen or seed transfer to native populations, could be established using local and distant genotypes, either singly or in planned mixtures.

It is usually assumed that establishing a new population from a local source must bring benefits in local adaptation. The existing experimental evidence indicates that local adaptation and co-adapted gene complexes break down only short distances away from the native locality and that some plants may have optimal outcrossing distances (Price & Waser 1979). For example, Waser & Price (1994) found that progeny of *Delphinium nelsoni* from intermediate crosses (3 m & 10 m distance from maternal plant) had greater growth and survival than close (1 m) or distant (100 m) crosses. Clearly in this case outbreeding and inbreeding effects were involved. The assumption must be that for this species the beneficial effect of

the co-adapted gene complexes breaks down much less than 100 m from the parent location. Although the scale of these effects will vary between species depending upon their breeding systems and the mechanisms of pollen dispersal, this research suggests that co-adapted gene complexes do not confer an advantage in the establishment of plants beyond a very local area. As the balance between inbreeding and outbreeding depression becomes better understood it is possible that the precautionary principle may shift away from using local material even for re-stocking existing populations.

Structure and size of populations and metapopulations

Increasing fragmentation of landscapes and the loss of plant populations means that the survivors are often now increasingly isolated. Where the species formerly existed as part of a metapopulation, local surviving populations are at increased risk of extinction, with loss of genetic variability possibly contributing to this risk (Kay & John 1994). Occasional gene flow from neighbouring populations may have helped maintain genetic variability and avoid inbreeding depression without necessarily compromising local adaptation (Ellstrand 1992). It is unlikely that any species would benefit from increasing isolation unless, like the Plymouth pear *Pyrus cordata* (Jackson 1995), it is threatened by introgression with a congener (Ellstrand 1992).

A significant number of recovery programmes aim to restore populations known, from historical records, to be lost. In doing so, conservation managers may be creating further isolated populations with a low probability of persistence. Quinn *et al.* (1994) recommended that when undertaking (re)introductions, managers should be trying to create clusters of populations within dispersal range of one another, thus increasing the effective population size overall (Ellstrand 1992). Occasional gene flow between the elements of a cluster can be sufficient to counter the effects of drift within small, isolated populations. We endorse this approach, whether for creation of new populations or re-stocking of existing ones. It is especially important where meta-populations are known to have been destroyed or damaged (Bowles *et al.* 1993).

Guideline: new or restored populations should be created as clusters of populations to create or recreate functioning metapopulations.

Attention needs to be paid to genetics and breeding systems in this approach. Populations should be created or restored to an optimum density, to permit gene flow, matching that observed within viable native populations (Kunin 1992; Van Treuren *et al.* 1993; DeMauro 1994). Planting schemes may need to ensure that compatible mating types are grouped together or genotypes are spatially arranged to minimise inbreeding (DeMauro 1994; Jackson *et al.* this volume). Distances between the elements of a cluster should be guided by the breeding system of the species and the likely effective dispersal distances of pollen or seed (Ellstrand 1992). These decisions can be informed by what is known about former metapopulation dynamics (Bowles *et al.* 1993).

Newly created or augmented populations should be made as large as possible, within practical or habitat constraints, to counter extinction risk and losses at transplanting (Birkinshaw 1991; DeMauro 1994). Larger populations are also less likely to accumulate deleterious alleles through mutation which could significantly increase the chance of extinction (Lynch, Conery & Burger 1995). Guidelines to minimum effective population size may be useful in the absence of specific studies of viable and effective population size (Franklin 1980; Soulé 1980; Lande 1995). Given that gene flow by pollen is mainly from large

to small populations, and that the latter receive external pollen at higher rates than large populations (Ellstrand 1992), it may be possible to influence gene flow by manipulating the respective size of elements of a cluster.

Guideline: new or restored populations should be as large as possible within ecological and pragmatic constraints.

Many of the previous recommendations imply an experimental approach to plant recovery programmes. Such experimentation is vital to increase our understanding of the role of genetics in the performance of small populations. Recovery attempts offer an ideal opportunity to gather empirical data that can be applied more widely to the conservation of other threatened plants. Such experiments should be incorporated as an integral part of species action plans. Indeed, Pavlik (1994) stated that "under no circumstances should seeds or ramets be released into suitable habitat without the controlled statistically empowered design of a good experiment".

Guideline: all recovery attempts involving the genetic manipulation and screening of populations should follow rigorous scientific practice and be fully recorded in order to inform future programmes and increase fundamental knowledge.

Discussion

The imperative to initiate recovery programmes for threatened plants is invariably driven by demographic concerns (Pavlik 1994; Schemske *et al.* 1994). This is entirely appropriate. Demographic (and environmental) stochasticity are major threats to small populations. Equally, demographic data are relatively simple to gather and may be most readily available for assessing the risk of extinction. Yet there is increasing evidence, reviewed in part in this paper, that the genetic structure and history of populations may also have profound implications for the survival of populations. Indeed, the demographic justification for intervening in populations is often supported by concern over genetic factors (Nunney & Campbell 1993). For example, modelling suggests that the accumulation of deleterious alleles in small populations can contribute significantly to extinction risk (Lynch, Conery & Burger 1995). If we are to attempt to maintain or restore long-term viability to populations we need to take these issues seriously, but existing guidelines rarely address genetic issues in any detail.

Conservation managers need to be able to recognise the circumstances where consideration of genetic effects is most appropriate. This does not mean that potentially time-consuming or expensive genetic screening need always be carried out, although in some cases it will be vital. At the simplest level, avoiding unconscious anthropogenic selection pressures on populations being sampled, maintained *ex-situ* or established in the wild, is an important first step – but one that is often overlooked. A number of our guidelines, which can be readily incorporated into any programme, address this significant point. However, genetic concerns come to the fore when conservation managers choose to directly intervene in populations. Key questions that may then arise, such as what source of material should be used for translocations or re-stocking, begin to incorporate a genetic component. Yet most existing guidelines recommend the precautionary principle of using local genotypes. In some cases, however, such as for Lakeside daisy *Hymenoxys acaulis* var. *glabra* (DeMauro 1994), simply pursuing this precautionary approach would not have prevented the extinction of the population under investigation because it consisted only of sexually incompatible individuals. Determining and manipulating the genetic structure of that population was central to the attempt to restore full viability to the colony.

In developing these guidelines we have attempted to integrate recent research and theory on the genetics of small populations with the current principles and practice of plant recovery programmes, based largely on demography. It is our submission that the two approaches are entirely compatible and intimately linked. There are few steps in recovery programmes that do not potentially require some consideration of the implications of genetics even if the need for any specific action is then rejected. The guidelines presented here are intended to pragmatically address these, regardless in some cases of whether detailed genetic studies are available or not. We believe it is imperative that genetic implications are fully *considered* in planning and implementing recovery programmes. There is little justification for not doing so; indeed they may be critical for the long-term success of many recovery programmes.

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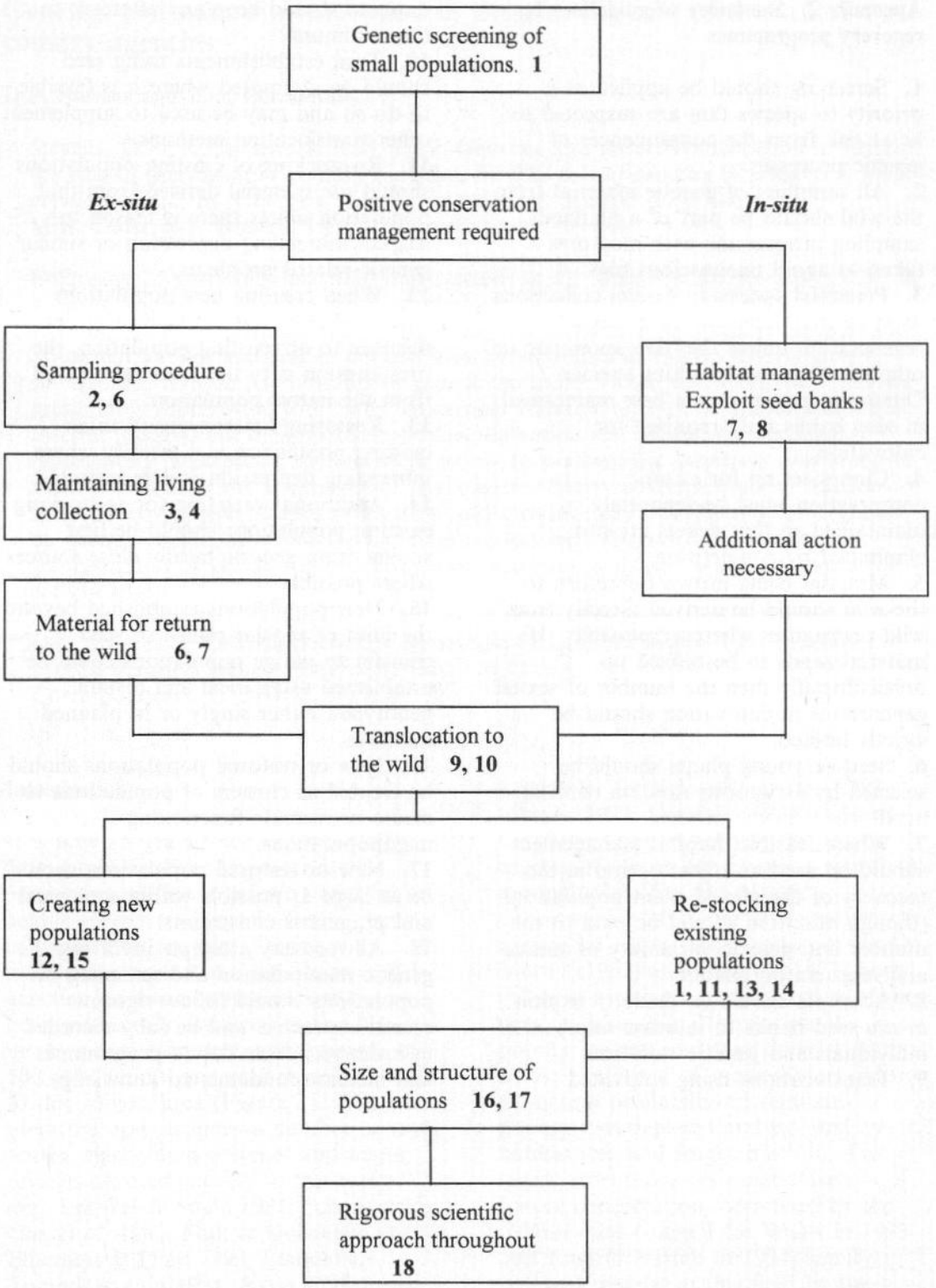

Appendix 1. Application of guidelines to recovery programmes (numbers in bold refer to guidelines summarised in Appendix 2).

Appendix 2. Summary of guidelines for recovery programmes

1. Screening should be applied as a priority to species that are suspected to be at risk from the consequences of genetic processes.
2. All sampling of genetic material from the wild should be part of a planned sampling programme with measures taken to avoid unconscious bias.
3. Perennial species in *ex-situ* collections must be maintained without sexual regeneration unless they are apomictic or otherwise rigorously selfing species. Outcrossing annuals are best maintained in seed banks until required for cultivation.
4. Clones set up for *ex-situ* conservation must be separately maintained so that genets are not eliminated by competition.
5. Material being grown for return to the wild should be derived directly from wild propagules wherever possible. If material needs to be bulked up horticulturally then the number of sexual generations in cultivation should be strictly limited.
6. Seed or young plants should be selected by a rigorous random method at all stages in the process.
7. Where feasible, habitat management should be used as a first option in the recovery of threatened plant populations (though attention should be paid to the number and genetic variability of source and regenerating plants).
8. Attempts should be made to exploit *in-situ* seed banks to increase numbers of individuals and genetic variation.
9. Translocations using cultivated material should keep any aftercare to the minimum.
10. Trial establishments using seed should be attempted where it is feasible to do so and may be used to supplement other translocation methods.
11. Re-stocking of existing populations should use material derived from that population unless there is reason to suspect inbreeding depression or similar genetic-related problems.
12. When creating new populations within regular seed or pollen transfer distance to an existing population, the presumption is to use material derived from the native population.
13. Restoring heterozygosity to an existing population is a priority where inbreeding depression is demonstrated.
14. Additional variation for re-stocking existing populations should be first sought from geographically close sources where possible.
15. New populations established beyond the limit of regular pollen or seed transfer to native populations could be established using local and distant genotypes, either singly or in planned mixtures.
16. New or restored populations should be created as clusters of populations to create or recreate functioning metapopulations.
17. New or restored populations should be as large as possible within ecological and pragmatic constraints.
18. All recovery attempts involving the genetic manipulation and screening of populations should follow rigorous scientific practice and be fully recorded in order to inform future programmes and increase fundamental knowledge.

Genetics and nature conservation in Britain: the role of the statutory country agencies

D.P. Stevens and T.H. Blackstock

Stevens, D.P., & Blackstock, T.H. 1997. Genetics and nature conservation in Britain: the role of the statutory country agencies. *In*: *The role of genetics in conserving small populations*, ed. by T.E. Tew, T.J. Crawford, J.W. Spencer, D.P. Stevens, M.B. Usher & J. Warren, 193–203. Peterborough, JNCC.

Following 25 years of conceptual development in the international literature, there is growing interest in genetic aspects of biodiversity conservation within the British statutory country agencies. The present account includes a preliminary consideration of current habitat and species conservation programmes in Britain from a genetic standpoint. Substantial amounts of genetic variation within many native species are presumably represented within the nationwide network of Sites of Special Scientific Interest (SSSIs), but the effectiveness of conservation site selection procedures for representing intraspecific variation has yet to be evaluated; a summary case study of *Vicia orobus* in Wales is presented. Management intervention projects in relation to species recovery and habitat restoration and creation may have considerable impact on genetic composition and require careful planning. Proposals for further incorporation of genetics into conservation activities in Britain are put forward for consideration.

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Introduction

It is now 25 years since Frankel (1970) first emphasised the importance of conserving sufficient genetic variation to enable species to adapt to future changes in their environments. During the intervening period, the significance of genetics for nature conservation has been extensively discussed in the international literature, and upwards of 500 publications per year currently relate to this subject area (Figure 1). The literature now includes a number of text books, symposium volumes and major reviews devoted entirely to this topic (e.g. Frankel & Soulé 1981; Schonewald-Cox *et al.* 1983; Falk & Holsinger 1991; Ellstrand & Elam 1993; Loeschke, Tomiuk & Jain 1994; Avise & Hamrick 1996) as well as several others in which conservation genetics is discussed in the broader context of conservation biology (e.g. Soulé & Wilcox 1980; Soulé 1987; Simberloff 1988; Shafer 1990; Seitz & Loeschcke 1991; Fiedler & Jain 1992; Hansen 1992; Caughley 1994). Among the earliest papers relevant to genetic aspects of nature conservation were two written by staff of the Nature Conservancy (NC), the then statutory agency for nature conservation in Britain. Moore (1962) and Hooper (1971) both included a preliminary discussion of the concept of viability in relation to population size and they drew attention to the likelihood of inbreeding increasing extinction probability in remnant populations depleted and isolated by habitat loss and fragmentation. Two recent workshops on genetic aspects of nature conservation, organised by the Countryside Council for Wales in 1993 and English Nature in 1994, signify renewed interest in this field by the British conservation agencies. The agencies have recently commissioned genetic surveys of various species, several of which are described in the present volume.

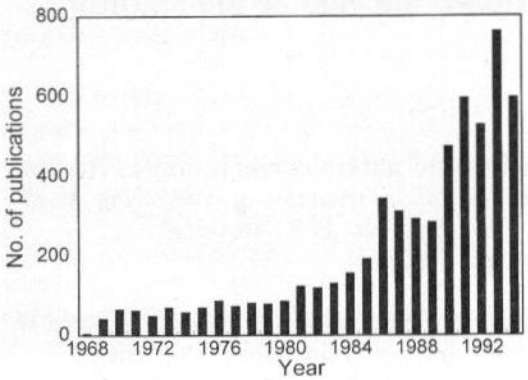

Figure 1 Expansion of literature on conservation genetics since 1968. Data are derived from a computer search of File 5 in the BIOSYS bibliographic database in June 1995. For each year, the number of publications with major concept codes relating to both conservation/ wildlife management and classical/population genetics is shown.

The papers presented at the York symposium integrate the conceptual framework of conservation genetics with case studies which demonstrate practical applications of genetic research. In this concluding contribution, current programmes for habitat and species conservation in Britain are considered from a genetic standpoint, focusing on the effectiveness of (1) site safeguard, (2) species recovery and (3) habitat restoration protocols for maintaining variation within native species. It is concluded that traditional conservation measures (designation and sympathetic management of key sites) are likely to favour retention of genetic variation in many species, but that emerging initiatives in restoration ecology raise potential hazards as well as new opportunities for genetic conservation. Outline proposals for raising the profile of genetics in biodiversity conservation programmes are put forward for consideration and discussion.

Site safeguard

Many species from a wide range of taxonomic groups have experienced severe declines in Britain during the present century and previously (Table 1). Destruction of semi-natural habitats, caused by agricultural intensification, afforestation and other land-use changes, has been identified as the most important influencing factor, although pollution, over-exploitation, persecution and displacement by introduced taxa have also affected some species (Ratcliffe 1984). Habitat destruction has been

Table 1. Examples of recent population decline in Britain's flora and fauna.

Taxonomic group	Scope of impact	Extent and period of decline*	Reference
Vascular plants	214/254 scarce species	Greater than 25% decline in pre–1970 records by 1992	Stewart, Pearman & Preston (1994)
Birds	43/237 breeding species	Greater than 25% decline between 1968 and 1993	Avery *et al.* (1994)
Butterflies	22/62 native species	Major range contraction over last 150 years	Heath, Pollard & Thomas (1984)

*Based on 10 km × 10 km square frequency for vascular plants and butterflies, population census data for birds. The former criterion is likely to under-represent population decline for many species.

Table 2. Summary data on recent habitat loss in Britain.

Habitat	Scope of study*	Extent and period of decline	Reference
Lowland grassland	E & W	97% loss between 1930 and 1984	Fuller (1987)
Lowland heathland	E (6 districts)	40% loss between 1950 and 1984	Nature Conservancy Council (1984)
Lowland raised mires	E & S (5 districts)	60% loss or damage between 1948 and 1978	Nature Conservancy Council (1984)
Ancient semi-natural woodland	E & W	45% loss between *c.* 1930 and 1992	Spencer & Kirby (1992)
Saltmarsh	E,S & W	11% loss between 1954 and 1984	Dijkema *et al.* (1984)
Upland heath	E & W	20% loss between 1947 and 1980	Thompson *et al.* (1995)

*E = England; S = Scotland; W = Wales.

non-random, with more extensive losses in the agricultural lowlands than in the uplands (Table 2). Genetic losses sustained by species dependent on habitats such as lowland grassland have not been estimated, but are probably substantial for some species. Ehrlich (1988) considered extirpation of genetically distinct populations to be just as severe a problem globally as species extinction.

For nearly half a century, one of the chief conservation responses to the problem of dwindling British species and habitats has been the safeguarding of key sites. The NC was empowered by the National Parks & Countryside Act 1949 to notify areas of land important for their flora or fauna as Sites of Special Scientific Interest (SSSI), a designation that was given additional protective measures by the Wildlife & Countryside Act (WCA) 1981. The statutory powers were subsequently transferred to the Nature Conservancy Council (NCC) and thence to the current country conservation agencies (Countryside Council for Wales, English Nature and Scottish Natural Heritage). By November 1994, 4762 SSSIs had been notified for biological features in England, Wales and Scotland, amounting to 18,779 km^2 or 8% of the total land area of Britain (Table 3). Individual sites vary considerably in area, and a recent analysis in Wales has quantified the contrasting numbers and sizes of sites in upland (few, large), lowland (many, small) and coastal (intermediate) landscape zones (Table 4). Most of these sites are selected as habitat examples, with about 40% qualifying on the basis of populations of one or more rare or declining species (Table 5). Several other conservation designations exist, but the SSSI mechanism remains the mainstay of site-based nature conservation in Britain.

The philosophy behind key site designation in Britain is outlined in two important publications. Command 7122 (Ministry of Town & Country Planning 1947) includes population genetics ('breeding structures of populations and the way in which inherited variations are distributed') as one component of the scientific interest to be considered in selecting a series of National Nature Reserves (NNRs). However, conservation imperative has been too great to allow genetic considerations to be taken fully into account in site selection. In a subsequent review of British NNRs and other high-grade conservation sites, Ratcliffe (1977) acknowledged that intraspecific genetic diversity had not determined site selection, but he anticipated that a wide range of genetic variation within native

Table 3. Biological SSSIs in Britain designated between 1949 and 1994.

	No. of sites	Total SSSI area (ha)	% land area notified
England	2973	852894	6.5
Wales	731	202521	9.8
Scotland	1058	822472	10.6
Britain	4762	1877889	8.0

Table 4. Numbers and sizes of biological SSSIs in upland, lowland and coastal landscape zones of Wales. Position in November 1994; data from Blackstock, Stevens & Howe (1996).

Landscape zone	No. of sites	Total, area ha	Site area, ha (median and range)
Upland	63	122637	234 (4–22770)
Lowland (inland)	555	24439	13 (1–1881)
Coastal	113	55445	76 (1–10704)

Table 5. Numbers of biological SSSIs in Wales designated for habitats and species. Position in November 1994; data from Blackstock, Stevens & Howe (1996). Total no. of sites: 731.

		Habitats	
		+	–
Species	+	269	59
	–	394	9

species had been incidentally included in the series of sites chosen primarily for habitat representation.

Current principles and procedures for selecting SSSIs are outlined in *Guidelines for selection of biological SSSIs* (Nature Conservancy Council 1989, with subsequent supplements). In this document, emphasis is attached to habitat area and population size for site evaluation, and mention is made of the genetic problems of inbreeding in very small isolated populations. Another important guiding principle is that sites are selected to cover the full range of habitats and species of conservation value within roughly county-sized 'Areas of Search'. This means that country-wide variation within individual habitats and species determined by climatic and other macro-environmental variables should automatically be accommodated within the SSSI network.

From a conservation genetic standpoint, the SSSI selection procedures appear to go some way towards representing intraspecific variation. Most characteristic species of semi-natural habitats should be represented in a number of SSSIs distributed over a wide geographical range. Rare and declining species may be additionally represented on sites supporting important local populations. Substantial amounts of genetic variation within many native species are therefore presumably represented within the SSSI series. However, the effectiveness of the SSSI procedures for capturing intraspecific genetic variation in native species has yet to be evaluated.

For a given species, assessment of genetic representation within a network of conservation sites could be carried out at two levels. Firstly, the potential representation of genetic variation could be assessed by considering the extent to which the geographic and ecological distributions of the species are sampled in the SSSI series, effectively using these as surrogate measures of genetic patterning (see also Gray this volume). Secondly, the *actual* representation of genetic variation could be assessed if adequate and reliable genetic survey data are available. A preliminary attempt to carry out such assessments for Welsh populations of wood bitter-vetch *Vicia orobus* in biological SSSIs is included in Box 1. The genetic analysis in this example is based on isoenzyme data, but assessment could equally make use of molecular, cytogenetic or biometric characteristics. Appropriate methods for describing patterns of variation for conservation application are still being debated (Schaal, Leverich & Rogstad 1991; Ennos *et al.* this volume).

By developing this approach, it may be possible to assess the extent to which inter-population variation within species of conservation concern is represented in protected sites. Moreover, by building up a series of assessments for species representative of different taxonomic and functional groups (e.g. inbreeding plants, mobile vertebrates, sedentary insects), it may be possible to evaluate the effectiveness of the SSSI system for representing intraspecific variation in native British wildlife in general.

Species recovery and *ex-situ* conservation

Although site safeguard continues to provide the main mechanism for delivering statutory nature conservation in Britain, there is a growing realisation that for some endangered species simply protecting remnant populations is not enough. For these species, management intervention to recover geographical range and population size is required.

Two nationwide programmes of species action or recovery have recently been developed by the British conservation agencies. Conservation and recovery plans for the species then afforded full protection on schedules 5 and 8 of the WCA 1981 were produced by Whitten (1990), and action plans for a wider range of species are currently being

Box 1 Geographical, ecological and genetic characteristics of protected populations of *Vicia orobus* in Wales.

Wood bitter-vetch *Vicia orobus* is an insect-pollinated perennial legume with little capacity for vegetative spread. It has a strongly Atlantic distribution in Europe, with a major British stronghold in Wales. It is a declining species in Britain, and most of its Welsh populations are small (less than 100 individuals, rarely more than 1000). It is largely confined to dry acidic and neutral grassland in the lowlands, extending locally into wetter *Molinia* pasture. It also occurs peripherally in patches of scrub and bracken as well as on hedgebanks, road verges and streamsides. Populations of *V. orobus* are represented in 33 biological Sites of Special Scientific Interest (SSSIs) in Wales. The following comments provide a preliminary assessment of the extent to which geographical and ecological range and genetic (isoenzyme) variation are captured within the SSSI series.

Geographical and ecological range

The geographical and ecological distributions of protected and unprotected populations of *Vicia orobus* are shown in Figures A and B. Populations are represented within SSSIs throughout most of the geographical range of the species in Wales, apart from Pembrokeshire in the south-west where it is largely confined to isolated colonies on hedgebanks, road verges and coastal cliff-tops. Populations in acidic, neutral and wet grassland, as well as other habitats such as bracken and scrub, are represented within the SSSI series.

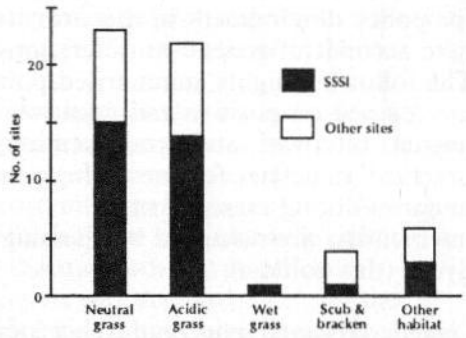

Figure B Ecological distribution of *Vicia orobus* populations in Wales showing representation in biological SSSIs. Data are summarised from records made during CCW's lowland grassland survey between 1987 and 1995 and are based on plant communities described in the National Vegetation Classification (Rodwell 1991 *et seq.*).

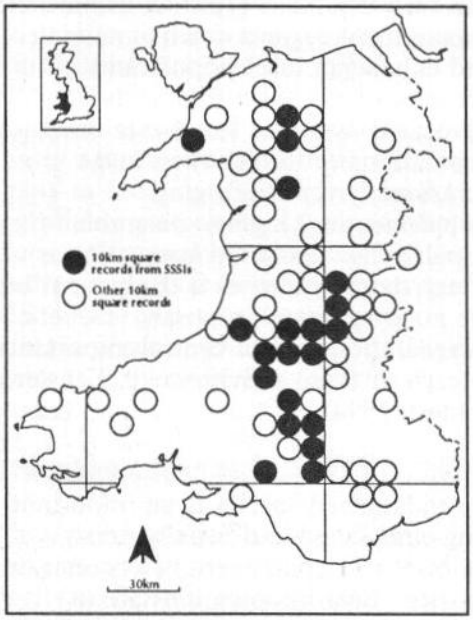

Figure A Distribution of *Vicia orobus* in Wales showing 10km squares in which populations are represented in biological SSSIs. In parts of mid and north Wales, up to six SSSIs with *V. orobus* occur within the same 10km square. Only post-1970 records are included.

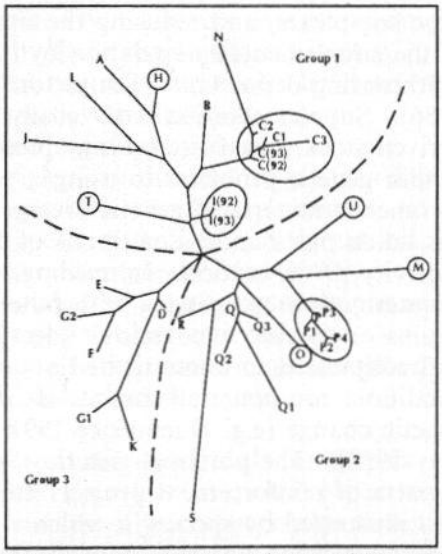

Figure C Unrooted phylogenetic tree for 21 populations samples of *Vicia orobus* in Wales showing three main genetic groups (Kay & John 1994). Encircled populations are located within SSSIs. Populations C,G,P and Q were subsampled, and populations C and I were sampled in both 1992 and 1993.

Isoenzyme variation

A survey of isoenzyme variation in *Vicia orobus* was carried out for the Countryside Council for Wales (CCW) by Kay & John (1994) as part of a broader investigation of genetic variation and reproductive biology in scarce and declining vascular plants (Kay & John this volume). Populations of *V. orobus* were sampled from 21 sites, including eight SSSIs; sample size varied from five to 88 with a mean of 24. Nine genetic loci with a total of 34 alleles were assayed. High levels of local differentiation were reported, with 29% of the overall variation distributed among populations. An unrooted phylogenetic tree was prepared in which branch length is proportional to the genetic distance between population samples, and three broad genetic groups were recognised (Figure C). Two of these three groups are represented by populations in protected sites. The third group comprises four populations from Pembrokeshire and one from Gwent, none of which is represented within the SSSI series.

Conclusions

This preliminary assessment indicates that SSSI selection has been partially successful in representing the geographical and ecological range of *Vicia orobus* in Wales and in capturing genetic (isoenzyme) variation among its populations. It also draws attention to the significance of the Pembrokeshire populations for representing the full range of variation within the species.

prepared as part of the UK response to the 1992 Biodiversity Convention. These and other related programmes provide a structure for developing a more planned and co-ordinated approach to species management.

Conservation measures in species action and recovery plans typically include site management regimes for maintaining and enhancing local populations. Translocation is also frequently advocated as a tool for re-establishing populations at former sites, and occasionally for reinforcing (supplementing) highly vulnerable populations. *Ex-situ* conservation is often recommended as a component of the main recovery proposals. Genetic considerations are of central importance to each of these activities (e.g. Fenster & Dudash 1994).

Provision of favourable site conditions for endangered species is an important, long-established and usually non-controversial component of conservation activity. Small populations are at greater risk of extinction than their larger counterparts for a variety of genetic, demographic and environmental reasons (Shaffer 1981), so that population expansion will normally favour population persistence. Population viability analysis may help to assess critical life history stages for some high-profile species, but it is becoming increasingly accepted that no generic guidelines for minimum viable population size can be advocated for use in practical conservation programmes (e.g. Soulé 1987; Templeton 1994). In reality, populations of many endangered species are often so small that any increase in size is likely to be favourable to maintaining genetic variation within the species.

Proposals for re-introducing species to formerly occupied sites and for reinforcing dwindling populations have attracted much greater controversy, partly for genetic reasons. Many translocations in the past have been poorly documented leading to confusion over native distributions of species in some taxonomic groups (e.g. Oates & Warren 1990). In response to these concerns, many position statements, codes of conduct and guidelines have been developed by conservation organisations and wildlife societies over the last 30 years. On behalf of the British conservation agencies, the Joint Nature Conservation Committee is currently co-ordinating a systematic review of translocation policy, with wide-ranging contracted advice from the Institute of Terrestrial Ecology at Furzebrook (Bullock, Hodder & Manchester 1995). There is a clear need for policy development in this area to take account of genetic considerations. The following highly summarised points are focused on positive and negative impacts of conservation translocation; practical guidelines for preserving genetic integrity during management intervention are discussed by Fleming & Sydes (this volume).

Genetic concerns over reinforcing local populations with extraneous source material have centred around the risk of swamping locally adapted gene pools, causing the breakdown of co-adapted gene complexes, and reducing the fitness of the supplemented population by 'outbreeding depression' (Templeton 1986). Supplementation with locally derived stock raised *ex situ* may present similar genetic problems to using extraneous material if genetic divergence has taken place under conditions of captivity or cultivation. Inbreeding, contamination by crossing with other strains or species, unconscious selection and adaptation to domesticated conditions are potential sources of genetic change (e.g. Huennecke 1991; Kay 1993). The potential genetic impacts of reinforcement projects are well illustrated by species in which supplementation has been carried out for reasons other than conservation. For example, genetic swamping caused by deliberate and accidental releases of hatchery-reared brown trout *Salmo*

trutta and other salmonids is causing widespread concern among fish conservationists (Maitland 1992; Hansen & Loeschcke 1994; Ryman, Utter & Hindar 1995). Many supplementation proposals are therefore unlikely to be favoured from a conservation genetic standpoint. However, it may be justified to reinforce a population that is so small as to be in imminent danger of extinction, as was the case with tufted saxifrage *Saxifraga cespitosa* in north Wales (Parker 1982), or to translocate extraneous genetic material into a moribund population declining as a result of inbreeding depression. No convincing case for the latter form of genetic management appears to have yet been made for any species in Britain.

Re-introduction proposals for species with restricted dispersal capabilities raise fewer genetic concerns than reinforcement projects, as long as source populations are not adversely affected and the translocation is officially approved and documented. On the positive side, re-introductions may help to offset genetic losses caused by local extinction, because evolutionary divergence of the newly established population is almost inevitable (Barrett & Kohn 1991). In this context, mixing of individuals from different source populations may sometimes be justified, because the disadvantages of outbreeding depression may be outweighed by the advantages of a flush of variation from which particular genotypes can be favoured by natural selection (Templeton *et al.* 1990). Procedures for identifying appropriate donor populations and choosing individuals for transfer require further appraisal and development.

Re-introduction proposals for species with well-developed dispersal capacities raise similar genetic concerns to those of supplementation, because translocated individuals or their descendants may spread and cross with locally adapted populations. For example, if English populations of red kite *Milvus milvus*, re-established primarily from continental sources, spread into Wales, will they swamp or introgress the only remaining native British genotypes, or will they inject variation into an evolutionarily moribund population (see Cordero, Evans & Parkin this volume).

The lead role for *ex-situ* conservation of British native species is normally taken by institutions such as the Royal Botanic Garden, Kew and the Zoological Society of London. The country agencies have an important role to play in focusing *ex-situ* conservation programmes on priority taxa and also in ensuring that seed banking, captive breeding and other *ex-situ* programmes include measures to represent and maintain genetic variation within target species (e.g. Fleming & Sydes this volume).

Habitat restoration and creation

Although conservation of remaining semi-natural habitat remains the chief priority in Britain, increasing attention is currently being given by conservationists to habitat restoration and creation as a means of ameliorating the impacts of habitat fragmentation (Kirby 1995). There are now unprecedented opportunities for enhancing nature conservation on agricultural land, around 20% of which may be surplus to UK food production requirements by the year 2000 (Green & Burnham 1989). Changes in countryside policy are favouring the management of farmland for environmental benefit, and are implemented by a wide range of agri-environment schemes now available to farmers and other land-owners in many parts of Britain.

Limitations on what can be achieved through habitat creation on agricultural land are imposed by technical problems of regenerating appropriate types of land cover, in particular because of altered soil conditions under intensive management (e.g. Marrs 1993). It is also important to distinguish between

habitat creation carried out primarily for aesthetic reasons with that for which nature conservation is the main concern (Newbold 1989). In this context, gains for nature conservation may be best achieved by establishing favourable environmental conditions around or between existing conservation sites and allowing recolonisation to take place with minimum intervention.

From a genetic standpoint, well-planned habitat restoration and creation schemes may have a positive impact on genetic variation within dependent species. A particularly valuable role of targeted schemes may lie in increasing the carrying capacity of remnant habitat patches by patch expansion. Another important function may be in restoring previous levels of dispersal and gene flow between recently isolated habitat patches via 'corridors' or 'stepping stones'. In each case, enhanced metapopulation dynamics may favour long-term viability for some species.

Conversely, poorly planned schemes may have negative impacts on genetic diversity. Re-connection of habitat patches isolated for long periods of time may result in genetic mixing of locally adapted stock, causing similar problems to those arising through supplementation (see above). For example, the recent construction of a fish pass at the Conwy Falls in Gwynedd, north Wales, has raised concerns about crossing between genetically differentiated migratory and resident populations of *Salmo trutta* (Marshall, Beaumont & Wyatt 1992). Genetic mixing may also occur if extraneous sources of plant material are used for revegetating land under restoration. Much discussion has centred around the use of 'wild flower' seed mixtures which frequently include alien forms of native British species (Akeroyd 1994). Similar concerns have been expressed for tree species, including Scots pine *Pinus sylvestris* in which analysis of monoterpene variation has led to the recognition of local genetic provenances (Kinloch, Westfall & Forrest 1986; Forestry Commission 1989).

Genetic survey data are likely to play an increasingly important role in habitat restoration and creation studies, particularly for estimating recent levels of inter-patch gene flow in species with contrasting dispersal capabilities prior to management intervention (Ellstrand & Elam 1993).

Discussion

Genetic considerations have traditionally been treated as secondary in importance in habitat and species conservation programmes in Britain. It has generally been assumed that by safeguarding a wide range of key sites for wildlife and maintaining species distributions, genetic variation within native species will be adequately conserved. Conservation imperative has been too great to await the arrival of genetic survey data and more precise guidance for effective genetic conservation. With the development of refined methods for screening populations for genetic variation, the time is now ripe for more widespread and rigorous application of genetic principles and data in conservation policies and protocols. This is a field in which scientific investigation has a potentially important role to play in influencing conservation practice.

It is important for conservationists to identify species at greatest risk from genetic impoverishment, so that priorities for research and conservation effort can be assigned. Taxa undergoing range fragmentation and population decline are likely to be most susceptible in this regard (Ellstrand & Elam 1993; Kay & John this volume). Within this group, taxa with restricted international distributions and world resource concentrations in Britain constitute a particular responsibility, because British populations may include a substantial fraction of their overall genetic variation. Other important categories

Table 6. Genetics as a component of biodiversity conservation.

UN Biodiversity Convention, 1992. Article 2. Use of terms

> "Biological diversity . . .. includes diversity within species, between species and of ecosystems."

Biodiversity: the UK action plan (Anon. 1994). Paragraph 3.17

> ". . .. within species variation has great significance as a component of biodiversity, both as an expression of the complexity of the living world and because it is a crucial property of species which enables them to change and respond to their surroundings."

include taxa with disjunct relict distributions, which are often associated with high levels of inter-population divergence and local adaptation, and edge-of-range populations, which may be closely adapted to extreme environmental conditions (Hoffman & Parsons 1991; Lesica & Allendorf 1995).

Formal recognition that genetic variation within species forms a fundamental component of biodiversity was given at the 1992 United Nations Convention on Biological Diversity in Rio de Janeiro, and has subsequently been confirmed by the UK Government in its initial policy response to the Rio Convention (Anonymous 1994) (Table 6). There is a need for a more informed view of genetic considerations in conservation practice, and the following outline proposals indicate some ways in which this might be achieved in Britain. They are presented with the preceding summary of conservation programmes in mind and draw on conclusions and discussion points presented at the York symposium. Inevitably, the list is incomplete and provisional.

- Incorporate maintenance of genetic variation within and between populations of native species as an explicit aim in biodiversity conservation.
- Ensure that policies and guidelines for site safeguard, species recovery, habitat restoration and *ex-situ* conservation are developed according to sound genetic principles.
- Identify priority taxa for specific attention, including those potentially at risk from genetic impoverishment.
- Commission applied genetic surveys of priority species using appropriate methods, as fund availability allows.
- Review the extent to which genetic variation in priority species is represented within protected sites, and consider whether management intervention is desirable.
- Encourage funding of innovative research on the consequences of genetic impoverishment for population viability, and promote the development of further generalised guidelines applicable to practical nature conservation.

It is hoped that these proposals will stimulate debate among conservationists. They also serve to emphasise the important role that geneticists have in providing research findings and advice for use in nature conservation practice. Conservationists continue to be faced with the difficulty of partitioning limited resources and evaluating the priority for genetic surveys.

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