The role of genetics in conserving small populations

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Foreword

How important is genetics to conservation biology? Graham Caughley's last paper (Caughley 1994) spoke for many in arguing that for most endangered populations we need to focus on their ecology and on the specifics of human threats (through over-exploitation or other aspects of increasing human numbers), rather than genetics. In more technical terms, he suggested we should mainly be worrying about environmental effects and demographic stochasticity, and less about diminishing genetic variability causing inbreeding depression or lack of adaptability.

I think Caughley's view (which was less simple than the above caricature) is broadly correct in the short-term. But even in the short-term, a principal effect of most human activity is increasingly to fragment populations. Questions about the genetic sub-structuring of the resulting metapopulations – as many papers in this volume show – cannot then be avoided. Some of the chapters discuss the interplay between population structure and genetic aspects of conservation biology in general terms, from both theoretical and practical management points of view, while others give concrete examples.

Some of these questions are highlighted in the chapter by Quentin Kay and Rosemary John on Welsh plants. The UK does not have many endemics (whose global survival necessarily depends on UK efforts alone) but, as these authors emphasise with explicit examples, "isolated local populations of rare plant species often have patterns of variation which are of great interest" to both theorists and conservation practitioners.

Genetic aspects of the conservation of small populations cannot be avoided in zoos, or in the *ex-situ* conservation of plants in botanical gardens (or, although less discussed and studied, in seed banks). This book has several interesting chapters focusing on such problems in botanical gardens. These studies have interesting similarities with, and differences from, the zoo studies that most people are more familiar with; it might be useful to pursue such similarities and differences more systematically.

Related to the problems of genetics of small populations in zoos and botanical gardens are those of reintroducing species into the wild, a kind of halfway house between *ex-situ* and *in-situ* conservation. In this regard, the chapter by Pedro Cordero, Ian Evans, David Parkin and Colin Galbraith on the reintroduction of red kites from Spain into southern England and from Sweden into northern Scotland, outlining what they have done so far along with their plans for future monitoring, is particularly interesting.

I have pointed out that other well-intentioned schemes of introduction or reintroduction may have perverse consequences, if such considerations of local genetic differentiation are overlooked (May 1994). Re-planting hawthorn hedgerows in Wales with seeds from eastern Europe may be cheaper than using UK-collected seeds in the short run, but the long-term consequences are dubious from a conservation standpoint (and could cost more if the Polish genotypes do not like the Welsh weather). By the same token, Eminent Personages scattering wildflower mixtures along roadsides is all very well if your view of 'wildflowers' is shaped by Victorian chocolate box tops, but a great deal less sensible if you are aware of the realities of local varieties and the structure of local gene pools.

Too often, the tough practicalities of genetic aspects of conserving small populations degenerate into formal, largely mathematical, studies reminiscent of the Glass Bead Games of Magister Ludi's world. Not so in this volume. Another common failing is an exclusive focus on the charismatic: birds, mammals, some spectacular plants. These organisms are wonderful in the most literal sense of the word, but they are not necessarily the front-line organisms for long-term sustainability of the biosphere. The present book has a better balance than the outcome of most symposia on this broad subject: five chapters on general ideas; 9.5 on plants (Michael Gillman and Jonathan Silvertown's discussion on the IUCN rules draws on both plant and animal data); three on birds and mammals; 1.5 on insects (but the insects are butterflies, and, as such, really honorary birds); and none on 'lower plants', fungi or other micro-organisms. All but one of the 14 chapters relating to specific organisms are terrestrial. Thus the book is well balanced for a work in this field, but at the same time it underlines the desirability of reappraising the balance of effort among classes of organisms.

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PREFACE

The influence of genetics on the development of nature conservation in Britain has thus far been marginal in comparison with the roles of ecology, biogeography and taxonomy. Of necessity, conservation effort has focused on habitat and species conservation measures, both on protected sites and in the wider countryside, and relatively little attention has been paid to the conservation of intraspecific genetic variation. Now, however, the scientific literature attests to a growing concern that species may be losing genetic variation, primarily as a result of habitat destruction and fragmentation, both by extirpation of distinct genotypes and by genetic drift within isolated remnant populations. Hybridisation and introgression between introduced species or races and native relatives may lead to further losses of genetic diversity.

There are several reasons why genetic depletion is of concern.

1. Loss of variation within species and populations may restrict their ability to evolve and adapt to changing environmental circumstances.
2. Inbreeding effects in small, isolated populations may impair reproductive fitness, thereby compounding demographic problems associated with small population size.
3. Distinctive variants that are (possibly uniquely) adapted to local or regional ecological conditions may be destroyed.
4. Long-established patterns of genetic variation between and within populations may be lost or disrupted.

With concerns such as these in mind, Professor Michael Usher suggested in May 1994 that the British Ecological Society (BES) might convene a symposium on genetic conservation in conjunction with the country conservation agencies [Countryside Council for Wales (CCW), English Nature (EN) and Scottish Natural Heritage (SNH)] and their Joint Nature Conservation Committee (JNCC). An organising committee was convened, and the symposium was subsequently held as a joint meeting of the BES Ecological Genetics and Conservation Ecology Groups at the University of York on 18–19 September 1995. It was attended by over 100 delegates, and was the first international symposium concentrating on genetic aspects of nature conservation to be held in Britain.

The aim of the symposium was to bring together conservation practitioners and genetic researchers to explore the significance of genetics in conserving and managing small populations of plants and animals. Over the two days of the meeting, 18 invited papers were presented in five sessions, and these were supplemented by six offered poster presentations. Contributions varied from accounts of original research to short reviews and discussion pieces; case studies presented cover a wide range of taxa, including birds, mammals, butterflies and several vascular plant species.

Following the meeting, an editorial board oversaw production of the symposium proceedings. Each paper was refereed by two members of the editorial board and some by specialist external referees. All 18 papers are included in the present volume, together with summaries of three of the poster presentations.

It is appropriate to ask what messages emerge from the symposium that are of direct relevance to the conservation practitioner. Many recommendations and proposals are made in the individual papers, but here we highlight four broad points.

1. Genetic variation within species is a fundamental component of biodiversity, and its conservation should feature prominently in nature conservation programmes.

2. Conservation genetics is a complex and youthful science; population genetic theory and molecular methodologies are still evolving. This does not, however, diminish its importance.
3. Patterns of genetic variation within species differ according to their reproductive systems and population histories; these need to be taken into account in developing conservation strategies.
4. It is important to consider representation of genetic diversity in both *in-situ* protected sites and *ex-situ* collections. Conversely caution is required over the potentially negative consequences of translocations, particularly in relation to genetic mixing and disruption of long-established patterns of genetic variation.

We hope that the proceedings will stimulate a wider debate among British conservationists and conservation biologists. If this volume succeeds in promoting further integration of genetics into biodiversity conservation programmes, it will have achieved its main objective.

The editors
March 1996

Section 1 – Framing the questions

The expanding field of genetics is raising fundamental questions for society. Often, these questions go beyond the realms of science and into ethics. This conference, however, was concerned only with the scientific issues, leaving the ethical issues for others to debate.

In the field of nature conservation, policy-makers and practitioners are becoming increasingly aware of how genetics, and the conservation of genetic variation, raises a new suite of challenges. The first three papers in these proceedings seek to frame some of the questions that genetic science raises and place them in the overall context of nature conservation, with particular reference to Britain.

The first paper, by Michael Usher, looks at the problems faced by small populations and at some of the ecological theories which explain their circumstances. Habitat fragmentation, island biogeography, minimum viable populations and metapopulation theory are all considered. The potential genetic consequences of small or declining populations, and the value to biodiversity of small populations, are assessed. Michael Usher concludes that conservation of the genetic composition of small populations of species must be an important part of any effective Biodiversity Action Plan.

Sam Berry gives a personal viewpoint on the history and importance of conservation genetics. He argues for a holistic consideration of genetic influence and emphasises the importance of genetic architecture and stress for the underlying processes. Few of the other contributions to these proceedings consider these issues and, although this is partly because highly theoretical analyses are inappropriate for a publication such as this, it does suggest that the contribution of these processes to genetic variation is being overlooked. Sam Berry also urges conservationists to be clear about their motives, noting that "weakly anthropocentric" arguments are more honest, and may be even more valid, than 'strong anthropocentrism'.

In the third paper, Jonathan Spencer addresses the genetic information requirements for effective conservation advice. He concludes that there needs to be greater understanding amongst conservation officers of genetic concepts and the value of conserving both levels and patterns of genetic variation.

Thus, this opening section outlines some of the questions, both theoretical and practical, that conservationists should be asking when planning programmes to conserve biodiversity. There is an increasing realisation that conservation is a multidisciplinary activity. Management of a living natural resource requires ecological skills in order to form appropriate plans for that resource's maintenance and enhancement. However, the form of management may have profound genetic effects which are more likely to be manifest in the longer, rather than the shorter, term. Management therefore needs to be based on an understanding of the genetic effects, as well as including expertise relating to ecology, land management and environmental economics. It is only through such a multidisciplinary approach that we can hope for the biodiversity of Britain to be sustained for future generations.

Following the consideration of broad principles in the first section, the rest of these proceedings illustrate ways in which specific questions may be answered.

Small populations: fragmentation, population dynamics and population genetics

Michael B. Usher

Usher, M.B. 1997. Small populations: fragmentation, population dynamics and population genetics. *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, 11–21. Peterborough, JNCC.

In many parts of the world, formerly extensive habitats have become fragmented into many small remnants, although some habitats have always occurred in small, isolated units. This observation poses several fundamental questions. Is fragmentation important when planning the conservation of species and habitats, and are corridors between fragments effective? How are populations affected by fragmentation? Island biogeography might indicate that there would be fewer species in the remaining habitat patches. However, does movement around the landscape mean that more species can be maintained than would be predicted by island biogeography theory, or alternatively that the species complement is more dynamic because of the existence of metapopulations?

Should nature conservationists be concerned about the potential genetic effects of small populations? These include inbreeding, the loss of heterozygosity, founder effects and genetic drift. Consideration of a number of case studies indicates that it is likely to be difficult to predict whether any genetical consequences of small population size will become apparent. There are, therefore, still many important scientific questions to answer, and the genetic component to assessments of biodiversity tends to be disregarded or downplayed. Small populations, often occurring on habitat remnants, add considerably to local biodiversity. The application of population genetics, together with the application of population ecology, will be important in the practical management of these remnants and in attempts to enhance them.

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Introduction

Many problems in nature conservation revolve around the management of very small populations of species. The sort of question that is frequently asked is "How can I prevent the species going extinct?" For example, one population of oblong woodsia fern *Woodsia ilvensis*, monitored for the last 40 years in the Moffat Hills, Dumfriesshire, has steadily declined to the point where it will apparently go extinct imminently (see Figure 1); this is one of the few surviving populations of this species in Scotland, and all of them are very small. Would a knowledge of the genetics of this species have been helpful in reversing this long-term decline? As another example, in Holyrood Park in the centre of Edinburgh, the population of sticky catchfly *Lychnis viscaria* had declined by the 1980s to a handful of individuals. Because of the precipitous nature of its terrain it was impossible to search for every plant, though in the last three years (1993 to 1995 inclusive) it has been possible to see three, two and four plants, respectively, with flowers. A supplementation programme in 1994 used young plants from seed taken from one of the naturally occurring plants.

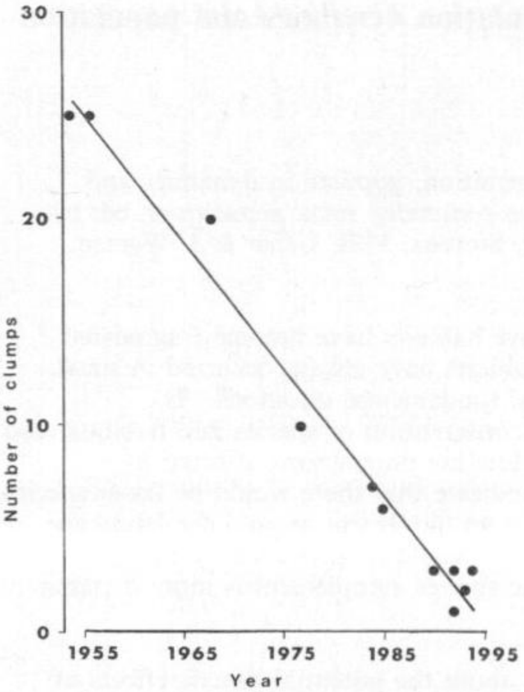

Figure 1. The size of one of the populations of oblong woodsia fern *Woodsia ilvensis* in the Moffat Hills. The regression line represents the equation $N = 1239 - 0.621Y$, where N is the number of clumps and Y is the year (this regression accounts for 97.9% of the variance). Data were provided by L.V. Fleming (pers. comm.).

The question that remains unanswered in this example is whether such an action is appropriate given the apparently small amount of genetical variation in the 'natural' population, or whether the introduced plants should have come from another site so as to increase the genetic variation locally.

These are examples of two species with very small populations where habitat fragmentation seems not to have been a factor. However, small, isolated populations are frequently induced by the fragmentation of once extensive habitats. The classic work of Moore (1987) showed how the once extensive Dorset heathlands became increasingly fragmented between 1896 and 1960, a process that has continued beyond the latter date (Anon. 1993). Fragmentation is undoubtedly a major factor affecting species because there is often no possibility that the sizes of depleted populations can increase again if the resources provided by the habitat patch are no longer sufficient. The subject of habitat fragmentation was addressed by Saunders *et al.* (1987), as well as by Saunders, Hobbs & Margules (1991).

The aim of this introductory chapter is to open up the topics addressed in the symposium, entitled *The role of genetics in conserving small populations*. The title indicates that the conference addressed many themes, namely:

- small populations (with the implied question of why they are small, whether rarity is in itself important, and what the dynamics of these populations are);
- conservation (implying the sustainability of the population over long time-scales, including both the need to consider metapopulation structures and habitat management to assist small populations to maintain themselves); and
- the genetical basis of such work (including the requirement to have a genetical appreciation of the dynamics of small populations and to understand the genetical implications of conservation management).

The conference therefore aimed to integrate scientific approaches to population genetics with the practical application of genetical research in conservation management and practice. This is all the more important with the recent publication of action plans designed to conserve, and enhance, the populations of some of the scarcest species in Britain (Anon. 1995).

Fragmentation

The subject of fragmentation raises several questions. How important is habitat fragmentation for species conservation? Is fragmentation a more serious threat for relatively non-mobile species than for more mobile species? In other words, how do species move around a landscape which is a mosaic of habitat patches, or can a species adapt to living within a single patch? If there is interchange of genetic material between the populations living in different patches, how can a landscape be designed and managed to support the metapopulation structure of those species? Do corridors assist in establishing a metapopulation structure, and, if so, how can they be designed to maximise that assistance?

Conservation values might be placed on habitat patches. But is a conservation value a count of the number of species present, perhaps with a weighting for the rare species, or does it include a component of the genetic diversity of the species present? Intuitively, small fragments have less conservation value than larger fragments, but it remains unknown whether this value decreases smoothly with decreasing size, or whether there are thresholds below which conservation value is lost. A series of possible models is shown in Figure 2. An indication that an abrupt, threshold model, model C, may be appropriate was gained in a study of farm woodlands, where woodlands of less than 1.5 ha appeared to have no characteristic woodland plant species and could be considered as totally woodland edge without woodland interior (Usher, Brown & Bedford 1992), although this might not be true for insects capable of flying (Dennis, Usher & Watt 1995).

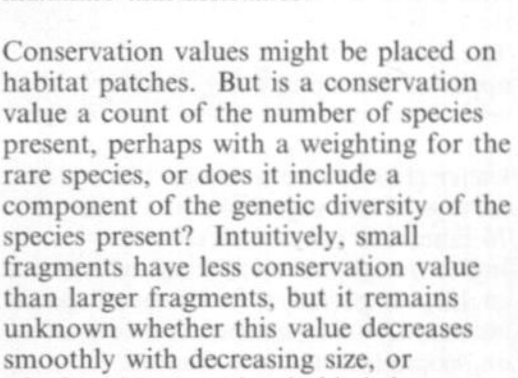

Figure 2. Possible models for the decrease in conservation value with decreasing size of fragment (re-drawn from Usher 1987). Model A indicates a gradual loss of value, whereas model B indicates a steeper loss of value between some range of sizes. Model C implies the existence of a single threshold, below which a fragment has very little conservation value.

There is a growing understanding of how species of animal and plant move around landscapes. Peterken's (1981) pioneering studies of woodland plants indicated very slow movement. FitzGibbon's (1993) study of grey squirrels indicated a number of factors, including proximity of suitable sites, that favoured this species, presumably by facilitating its movement across agricultural landscapes. Petit (1994) showed that a few individual carabid (ground) beetles use hedgerows to migrate from one woodland node to another up to a few hundred metres away, and from this she proposed a series of conservation guidelines for landscape management (Petit & Burel 1993). These studies do not consider the genetical implications of metapopulations connected together via landscape features such as hedgerows. This has, however, been studied by Wauters *et al.* (1994) for red squirrel populations. They demonstrated that genetic similarity among red squirrels is inversely correlated with population size in isolated populations as a result of reduced immigration.

Studies of this sort raise problems. Recovery programmes for some species that have declined to small numbers of individuals have been successful, whereas for other species no success has been achieved and genetical problems have been postulated. Wauters *et al.* (1994) concluded "What is also striking in our results is that the effects of demography have overcome the genetic consequences of finite populations, supporting the contention that ecological effects may be more significant than population genetics in determining the outcome of population fragmentation". This raises questions as to whether conservation practitioners need to know more about population dynamics than about genetics! The need to understand the dynamics of each individual population is further emphasised in the results of experiments on different levels of fragmentation (Robinson *et al.* 1992), where measures of plant and animal community diversity did not vary with the degree of fragmentation.

Island biogeography

The island biogeographical theory developed by MacArthur & Wilson (1967) is essentially concerned with the equilibrium reached between two ecological processes: the colonisation of an island by new propagules and the extinction of species already present on the island. When the rate of extinction equals the rate of immigration of new species, the equilibrium number of species will be maintained although the species complement of that number might change. Of course, with natural variability of climate, in any one year or run of years extinction may exceed immigration, or *vice versa*, but over a longer period of time the equality of the extinction and immigration rates will be maintained. Since the publication of MacArthur & Wilson's book a large literature in response to their ideas has developed, analysing a variety of islands and archipelagos, as well as habitat fragments, and there have even been some experimental studies, for example in the mangrove islands (Simberloff & Wilson 1969) which were defaunated by methyl bromide fumigation. These studies have not explored the genetical aspects of species turnover on islands or habitat fragments.

Many questions remain unanswered, however. Perhaps the greatest relates to metapopulations, occurring in habitat patches, but possibly with small 'stepping stones' connecting the 'islands'. This was part of the thinking of Diamond (1975) when he produced a simple model for how to design nature reserves. This model has been extended by Keiller & Usher (in press), explicitly incorporating connecting hedgerows and small habitat remnants, though they admit that much of their evidence is empirical rather than based on experiments. None of these designs for nature reserves takes cognisance of genetic factors, though implicitly it is accepted that if individuals can migrate between the individual populations in a metapopulation, then the genetical effects will 'look after themselves'!

The dynamic nature, either real or apparent (resulting from observer errors), of metapopulations is a feature of the results of Margules, Nicholls & Usher (1994), who analysed two surveys of the vegetation of a series of 74 limestone pavements around Ingleborough, northern England. Over an 11-year period, the number of species increased on 21 pavements, decreased on 46 pavements, and remained constant on the remaining seven pavements. However, looking at the rare and uncommon species which are locally dependent for their survival on the limestone pavement habitat, a very considerable amount of dynamism is apparent (Table 1). This is particularly important for biennial or annual species, such as *Cardamine impatiens*, which occurs on any one pavement in any one year as very few individuals (in general I have only found one individual on a pavement, and never more than 12), though presumably, being a crucifer, it has a reasonably large seed bank. Other species, such as *Asplenium viride* and *Cystopteris fragilis*, occur as large numbers of individuals. Again the genetical effects of such a dynamic metapopulation remain unknown, though with plants the seed bank needs to be considered because part of the genetical variability can remain *in situ* but unseen. Castillo (1994) considered that the soil seed bank of *Phacelia dubia*, a winter annual occurring in south-eastern USA, contributed in part to the preservation of the genetic

Table 1. 74 limestone pavements around Ingleborough, North Yorkshire, were surveyed in 1973/4 and again in 1985. For nine of the recorded species the table shows the number of pavements occupied in 1985, and the number of pavement extinction and colonisation events since 1973/4 (from Margules, Nicholls & Usher 1994).

Species	No. of pavements occupied in 1985	Extinctions since 1973/4	Colonisations since 1973/4
Rare species			
Actaea spicata	16	1	5
Cardamine impatiens	8	2	6
Daphne mezereum	0	1	0
Diplotaxis muralis	2	0	2
Dryopteris villarii	50	9	12
Uncommon species			
Asplenium viride	66	8	3
Cystopteris fragilis	73	1	0
Epipactis helleborine	1	3	0
Juniperus communis	3	2	2

diversity of this species, whose population size fluctuated year-to-year by three orders of magnitude. It also remains unknown whether all habitat fragments, such as these limestone pavements, would behave in an equally dynamic way, and whether invertebrate populations, for example, would be either more or less dynamic. As with studies of fragmentation, are the genetical implications of these naturally fragmented populations important, or is an understanding of population demography of greater importance?

Genetical consequences of small population size

Traditionally six genetic effects of small population size have been considered (e.g. Harris 1984; Usher 1987). These are:

- inbreeding,
- heterozygosity,
- founder effects (or 'bottlenecks'),
- effective population sizes,
- genetic drift,
- mutation rates.

Although there is relatively little information about mutation rates in wild populations, this is the only one of the six topics listed above that has the potential to increase genetic diversity. It is generally thought to be rather slow, and hence the prediction is that most small populations will be losing genetic variability. But, is this important?

Modelling exercises have generally predicted a loss of genetic variability (Lacy 1987) with genetic drift being the over-riding factor influencing vertebrate populations of a size commonly found in zoos and nature reserves. Lacy's simulations predicted that conservationists should manage their population so as to counter the effects of genetic drift, either by introducing very occasionally an immigrant or, and less effectively, by the division of the population into smaller breeding groups with interchange between the groups so that they do not suffer from inbreeding depression. Lacy's concept of occasional immigrants is theoretically attractive, but Wauters *et al.* (1994) showed that this was not effective in the red squirrel populations that they studied.

Each small population is different, and in some of them genetical effects might be demonstrated whereas in others there might be no apparent effect.

Experimental work is rare, but inferential conclusions based on observations are common. Leberg (1990, 1993) first used models to explore the effects of different numbers of founders (the more, the greater the amount of heterozygosity retained, as might be expected), but he followed this with experiments introducing mosquito fish to small mesocosms and observing rates of population increase. The experiments failed to give conclusive evidence, and indeed there is some suggestion that populations founded from two genetic stocks achieved lower sizes than populations founded from either one or other of the genetic stocks! The question remains as to whether these experiments provide evidence of outbreeding depression. There are apparently still large gaps between the models of theoreticians, the results of laboratory experiments, and observations in the field. Until the processes are better understood, it remains difficult to start thinking of applying theoretical and laboratory genetic concepts to the conservation of species richness in the field or sea.

Widén & Andersson (1993) examined field populations of *Senecio integrifolius*, a rare plant in Sweden. They claimed that their study was the first to relate quantitative genetic variation to population size in the field. Interestingly, they found far more genetic variability in a small population which consisted of several separate patches (an overall mean of 130 flowering plants per year, with one to 50 plants per patch) than in a large and more or less continuous population (a mean of 1260 flowering plants per year). However, a study of *Gentiana pneumonanthe* in The Netherlands (Raijmann *et al.* 1994) indicated that population size was positively correlated with the proportion of polymorphic loci but only marginally correlated with heterozygosity. They concluded that "because most small populations consist only of adult survivors from formerly large populations, this may partly explain the absence of a clear relationship between genetic variation of the maternal plants and population size". They did, however, indicate that higher levels of inbreeding in small populations of *G. pneumonanthe* contributed to a further loss of genetic variation and may also have resulted in reduced offspring fitness. The chapter by Prentice & Anderson (this volume) makes similar points about small *Silene diclinis* populations.

These few studies do not leave a clear picture of the importance to conservation practitioners of all of these genetic elements. The odds seem to be stacked against small populations – they will lose genetic variability by inbreeding depression, by loss of heterozygosity and by genetic drift – and only increase genetic variability by the rare mutant that is successful or possibly by immigration. The loss of genetic variability may, however, not be totally detrimental to the population; it is possible that deleterious genes may be eliminated. In practice it appears as if some small populations can, with appropriate management, increase satisfactorily, whereas others, such as *Woodsia ilvensis* in Figure 1, seem doomed to decline to extinction (the maxim seems to be: when a search for other factors fails, blame the extinction on genetics!). Perhaps it is safest to resort to the 'precautionary principle'; in complex situations accept that there may be genetical problems unless it can be proved that other factors are operative in reducing the size of the small population or preventing it responding positively under suitable conservation management programmes.

Small populations: rarity and the risk of extinction

It must be accepted that many populations are always going to be small; as Gaston (1994) showed, most species are rare, and this carries with it the implication that there are only small numbers of individuals of these species.

Rabinowitz's (1981) analysis of rarity is useful (Table 2); it could be argued that four of the seven categories, those with the word 'large' in the third column of Table 2, are not rare. Certainly, the focus of conservation attention tends to be on the three categories with the word 'small' in that column; how would the genetical effects, outlined in the previous section, affect species in these three categories?

For those species that are constantly sparse over a large range and in several habitats, there is a distinct possibility that there will be a metapopulation structure, with individuals or seeds (or pollen grains for flowering plants) moving between populations. Inbreeding and loss of heterozygosity are therefore likely to be reduced, though the effective size of any sub-population may be relatively small. For species that occur over a wide range in a specific habitat, the establishment or otherwise of a metapopulation structure will depend on the spatial distribution of the patches of the specific habitat and on the ability of each species to move between patches. With the loss of some patches, it may be difficult for individuals to move from one patch to another, and hence the metapopulation structure could more easily be broken. Inbreeding effects, the loss of heterozygosity and genetic drift all appear to be more probable.

However, it is in those sparse populations, occurring in a specific habitat in a restricted geographical range, that there is the greatest potential for genetic problems to arise. Rabinowitz (1981) suggested that many of these species may be endemics, though her studies of prairie grasses indicated that natural selection may operate to favour traits which offset the disadvantages of local small population size. She demonstrated that competitive abilities are crucial to persistence; is this another example of the ecological strategies of species being more important than the genetical properties of their populations?

Table 2. The seven forms of rarity postulated by Rabinowitz (1981). Note that there are three axes, relating to geographical range, habitat specificity and local population size. Rabinowitz suggested that one of the eight possible categories was non-existent.

Geographical range	Habitat specificity	Local population size	Population characteristics
Large	Wide	Large*	Locally abundant over a large range and in several habitats
Large	Wide	Small*	Constantly sparse over a large range and in several habitats
Large	Narrow	Large	Locally abundant over a large range and in a specific habitat
Large	Narrow	Small	Constantly sparse over a large range but in a specific habitat
Small	Wide	Large	Locally abundant in several habitats but with restricted range
Small	Narrow	Large	Locally abundant in a specific habitat and with restricted range
Small	Narrow	Small	Constantly sparse and geographically restricted in a specific habitat

* Large also tends to imply that the species is dominant somewhere; small carries the implication that the species is non-dominant wherever it occurs.

In the 1980s it became fashionable to consider that there must be some minimum size for a population, below which that population would in time experience difficulties resulting from loss of heterozygosity, loss of genetic variability, as well as demographic and environmental stochasticity (e.g. Soulé 1987). The concept of a 'Minimum Viable Population' size (MVP) was beguilingly simple. All one had to do was to determine the MVP, add on a margin for error, and then ensure that the managed population did not drop below that limit. Theoretically this was an attractive concept, but in practice it failed because the population sizes of many rare species were smaller than estimated MVPs, and indeed there are examples of some species that have recovered from very small numbers (as for example the Svalbard race of the barnacle goose *Branta leucopsis*, Figure 3). In Africa, Miller (1979) indicated that the population sizes of all large, wild mammals in protected areas were below the MVP, an observation that leads to the prediction that all these large mammals on the African continent will go extinct!

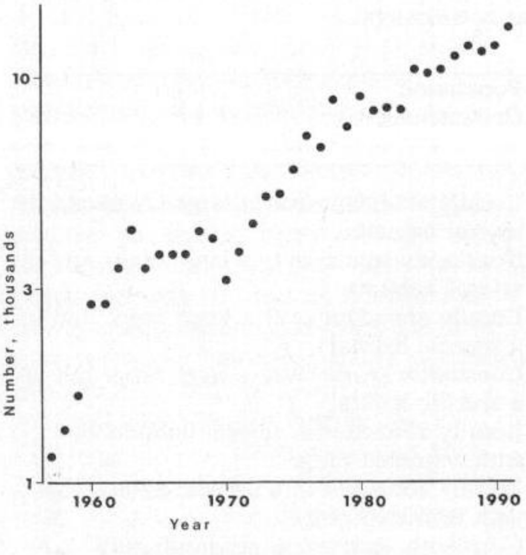

Figure 3. The recovery of the population of the Svalbard race of the barnacle goose *Branta leucopsis*. Points show the annual size of the overwintering population (in Britain) between 1957 and 1991. Data taken from Black (1995).

There are also two other words of warning about using MVPs. First, socio-economic factors have led to a number of people and pressure groups demanding reductions in populations of some rare species that are perceived to be threats to their livelihood, or just a nuisance. Although the expression 'Minimum Viable Population' is used, in reality what these pressure groups argue for is a 'Maximum Tolerable Population' (MTP). In the interests of reconciling conflicting interests, a species' future is possibly assured if the MTP is greater than the MVP, but real difficulties could arise if the MTP is less than the MVP. Second, defining an MVP may mean that species are managed at that level, without the margin for error. With year to year variability in population sizes, this means that the species could easily fluctuate below the MVP. The stability of the population will then become important: will it continue to fall, or will it increase again to the MVP? Perhaps, to apply the 'precautionary principle', we need to manage species populations at a size considerably higher than the MVP.

More recently, the focus of attention has moved away from MVPs towards risk assessments (e.g. Burgman, Ferson & Akçakaya 1993). Risk assessments are generally based on models and, as demonstrated in Burgman *et al.*'s book, seek to combine models of population demography with concepts of population genetics. The results are probably more realistic, indicating probabilities, under various scenarios, that a population will still be extant in, say, 100 years time. There is nothing deterministic in this approach, but the results are more difficult for a conservation manager to handle. The MVP approach gives a clear answer; maintain a population above X individuals and all is satisfactory (but is this clear answer the correct one?). The risk assessment approach gives a 'woolly' answer; maintain a population above X individuals and there is a less than 10% chance that it will have gone extinct within the next 100 years. Conservation managers in the future are likely to have to balance probabilities in an attempt to optimise their species or habitat management strategies rather than applying a set of rules of thumb. These risk assessments will continue to combine approaches of both population genetics and population ecology.

Discussion: protecting the biodiversity of small populations

Berry's (1971) paper to the 1970 symposium of the British Ecological Society was clearly an important milestone in introducing genetical thinking into conservation management. At the same symposium, Hooper (1971) also introduced some genetical concepts into the design of nature reserves, focusing mostly upon the problems of inbreeding. Now genetical effects are more widely recognised. Berry (this volume) traces the origin of his earlier paper and his subsequent involvement with 'conservation genetics' up to the present time. However, is it appropriate to separate 'conservation genetics' from other aspects of conservation, genetics and ecology? Do we need a holistic view of the science of conserving small populations?

In the 1990s the word 'biodiversity' tends to be replacing the word 'conservation'. Biodiversity can be considered at three levels, namely:

- genetic diversity,
- species diversity,
- diversity of habitats.

The benefit of this concept is that it embraces genetical considerations (the genetic level) and demographic considerations (the species level) with considerations about the structure and function of ecosystems (the habitats level). It is still true, however, that the genetic level receives little attention, except where developments in biotechnology are being considered (Stähler 1994). In recent texts on biodiversity, the genetical level gets little mention; for example Groombridge (1992) devoted six pages to the genetic level, 241 pages to the species level and 80 pages to the habitat level (chapters 1 to 24 inclusive), although Solbrig (1991) put forward a number of axioms and hypotheses at the genetical level (which he termed the 'molecular and cellular level'). If we are to understand the biodiversity of the planet, a nation, a region or even a very small local area, all three levels will need to be considered and researched. This presents difficulties, though more techniques are becoming available (e.g. Fleming & Aagaard 1993) for undertaking biodiversity inventories.

Small populations can contribute substantially to biodiversity and it is therefore important that their conservation is considered. There needs to be a greater understanding of the interplay of demographic and genetic factors. With increasing fragmentation of the landscape, more species are likely to occur in small patches of once more extensive tracts of their habitat. Harris' (1984) book highlights the fragmentation of woodlands, but it must not be forgotten that some habitats have always existed as small patches. An example of this is raised bogs. The processes since the last glaciation that have led to these small habitat patches are discussed by Lindsay (1995), but it is inevitable that the margins have been 'nibbled' into, and hence although there has probably been no fragmentation, the raised bogs of today are probably all smaller than they would have been 200 years ago. There is, therefore, a dynamic factor in the landscape; some habitats would naturally occur in large blocks whereas other habitats would naturally occur in small patches. The processes of land-use change will, in general, have led to the fragmentation of the large block habitats and to the miniaturisation of the small patch habitats. What effects have these processes had on the species that inhabit these habitats? Would Rabinowitz's (1981) contention that natural selection favours traits that offset the disadvantages of local small population size be more true of the species characteristic of small patch habitats than those of large block habitats? Would this imply that there is a greater chance of species extinction from fragmentation than from miniaturisation? There are many questions that remain unanswered.

Finally, are small populations worth conserving? Although there are many demographic and genetic problems associated with small populations, Lesica & Allendorf (1992) concluded that small populations, particularly those subjected to moderate levels of stress, may be more valuable for the conservation of biodiversity than would be predicted by models that assume selective neutrality. If small populations have this value, at both the genetic and species levels, then their conservation is important in any biodiversity action plan. This presents the managers of natural resources with a real challenge; how do we get into the 22nd century with at least the same biodiversity capital as we leave the 20th century (Usher 1991)? We need to look well into the future, and attempt to understand what our actions today will mean for people living in a hundred years time. Or, is that too short a time-scale as well?

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The history and importance of conservation genetics: one person's perspective

R.J. Berry

Berry, R.J. 1997. The history and importance of conservation genetics: one person's perspective. *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, 22–32. Peterborough, JNCC.

Conservation genetics is about 25 years old as a recognisable discipline. Over this period, the importance of genetics in ecology has been more clearly perceived and the two subjects have become more integrated. It is now accepted that ecological changes (or conservation management) are likely to produce genetic effects. Conservation geneticists have attempted to minimise the consequences of the latter, principally by advocating rules about the size of minimum viable populations. These rules have been easier to devise than apply, and fail to take into account the conservative influence of genetic architecture.

Conservation genetics *does* matter, because genetic diversity underlies and interacts with all other forms of diversity. But we have a long way to go before rational and general principles for management can be developed.

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Introduction

Conservationists have always been more concerned about the loss of habitats and of species than of genes; an extinct species or a destroyed habitat can be visualised and mourned much more easily than a gene, which is little more than a concept or a conundrum to most field biologists (by which I mean practising farmers or naturalists as distinct from academics (Holdgate 1994)). The beginnings of worry about genetic loss started from plant breeders with increasing replacement (and neglect) of traditional 'land races' by new varieties of cereals (particularly wheat and rice). This occurred around the same time as scientific interest was growing in the origin of crop species and the importance of ecotypic adaptation and differentiation. By the early 1970s this worry had become alarm, and action to identify and collect plant varieties was initiated through a section of the International Biological Programme and an FAO Panel of Experts (Frankel & Bennett 1970), leading to the establishment of a permanent International Board of Plant Genetic Resources (Williams 1988). Parallel concern and action took place in the zoological community (Rowlands 1964; Dobson *et al.* 1992).

These beginnings were largely pragmatic responses to a perceived assault on 'nature' (in the widest sense of that term). I was introduced to them about 1967 by Palmer Newbould, at that time responsible for the M.Sc. in Conservation established at University College London on the initiative of Max Nicholson, Director of the Nature Conservancy (Nicholson 1993). Newbould had been alerted to genetic problems by reading Charles Elton's *The ecology of invasions by animals and plants* (1958), particularly a chapter on 'the conservation of variety'. Elton was actually writing more with landscape than genetic variety in mind, but characteristic Eltonian ellipsis confused Newbould and fired him to introduce genetical conservation as a necessary part of the content of biological conservation. The importance of genetics in conservation was therefore a recognition by ecologists, not by the genetical community.

Thirty years ago there was no formal framework for a science of 'conservation genetics', and it was necessary to devise the subject from first principles: what consequence(s) does the reduction in the numbers in a population have for allele loss, inbreeding depression and differentiation (Figure 1)? I concluded (Berry 1971) that the simple probability of genetic change calculated from standard formulae over-estimated the likelihood of deleterious effects on the grounds that:

1. recurring mutation and recombination seem to provide abundant variation for environmental challenges;
2. selection is sufficiently common and strong normally to counteract random fluctuations in allele frequencies (genetic drift) in small (or fragmented) populations;
3. adaptation is rapid and precise;
4. inbreeding depression varies enormously from species to species, and cannot be predicted from homozygosity levels;
5. genomic and developmental interactions maintain many alleles in the gene pool despite the probability of their loss as calculated from simple statistical theory;
6. despite repeated claims in the literature about the dangers of random (non-adaptive) genetic changes, there was (and is) a lack of convincing examples of persistent drift (as opposed to intermittent drift due to bottlenecks in number) in natural populations (Berry *et al.* 1992).

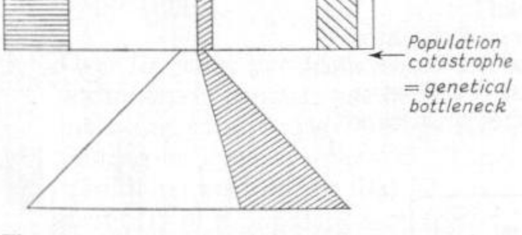

Figure 1. Any bottleneck in numbers may reduce genetic variation and change allele frequencies. A bottleneck may be the result of an ecological catastrophe, a colonising event, or the use of a small number of individuals to establish a domesticated race. Selection for particular traits may reduce variability even further.

The growth of conservation genetics

The past 25 years have witnessed an enormous growth and maturing of the subject of conservation genetics, particularly through the contributions of Michael Soulé (q.v. Soulé & Mills 1992). During this period, there have been a number of false enthusiasms and wrong emphases. For example, Soulé criticised my above conclusions on the ground that any loss of genetic variation reduces the potential for persistence and adaptation of a population or species; he categorised me as a 'phyletic optimist' (Frankel & Soulé 1981, p. 84). It may have been over-sanguine to assume that enough variation is always available (Berry & Bradshaw 1992), but I believe that Soulé's comments were over-stated and that both he and I were right in terms of conservation dialogue: the scientific evidence supports (I believe) my 'optimistic' judgement, but my stated conclusion that conservation practices were unlikely to produce significant deleterious genetic effects was an open invitation to policy-makers and managers to ignore the complex of factors which affects long-term viability and survival. Problems almost inevitably arise when management decisions are extrapolated too far from their justifying data; the danger is that the underlying science is doubted and falls into disrepute (Ashby 1980). However, conservation genetics has changed greatly since the 1970s. Avise (1996) lists five issues where genetics is currently empirically or conceptually important in conservation biology: heterozygosity (within population variability), parentage and kinship, population structure and intraspecific phylogeny, species boundaries and hybridisation phenomena, and species phylogenies. All these relate to the origin, maintenance and fate of variation (Figure 2), and illustrate how far the significance of genetics for conservation has progressed beyond the point where genetic considerations could be regarded merely as an interesting but unapproachable and probably irrelevant extension of conservation practice. Genetics cannot now be legitimately separated from ecological understanding or management.

The reasons for this are advances in both biological and conservation science, as follows.

1. In terms of general awareness, the inclusion of the preservation of genetic diversity as one of the three primary objectives of the *World Conservation Strategy* (IUCN, UNEP & WWF 1980, revised as *Caring for the Earth*, IUCN, UNEP & WWF 1991) was highly

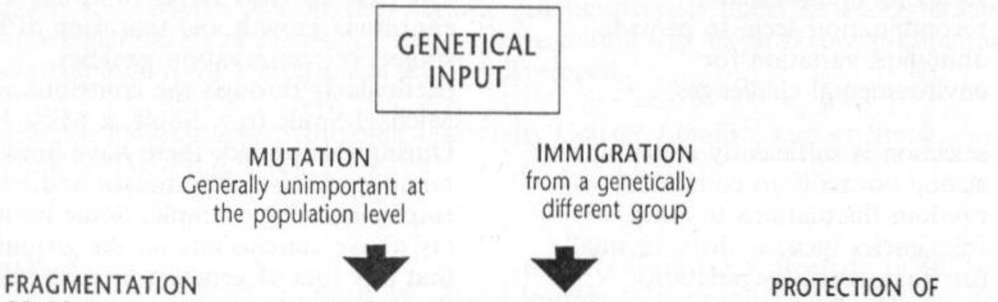

Figure 2. Genetic variation in a population is increased by mutation and immigration, and reduced by natural selection and drift; all these factors interact and all depend on the environment to some extent. A stressful environment will therefore affect the operation of these genetic forces. Note that ecological and developmental factors also have an impact (from Berry 1974).

significant. The Strategy stated that genetic diversity is both an insurance and an investment, "necessary to sustain and improve agricultural, forestry, and fisheries production, to keep open future options, as a buffer against harmful environmental change, and as the raw material for much scientific and industrial innovation".

2. The increased environmental awareness which has been a major stimulus for conservation action has brought also a questioning of our relationship to the natural world, and a growing recognition that we have a moral responsibility towards posterity and nature, as well as the strictly utilitarian interest expressed in the *World Conservation Strategy* (Black 1970; Elsdon 1992; Berry 1993). Although we are managers and apart from nature, we are also a part of nature and inextricably bound up with it. Frankel & Soulé (1981, p. 8) express this forcefully: "There is a growing feeling that to end all evolution . . . is an arrogant if not fatal step for man to undertake, and that as biologists we bear a special responsibility. No longer can we claim evolutionary innocence. We are still subject to evolutionary processes . . . but we are also major operators. We are not the equivalent of an ice age or a rise in sea level: we are capable of prediction and control. We have acquired evolutionary responsibility."

3. Over the same period that conservation genetics has been maturing, evolutionary biology has undergone a major upheaval. The traditional assumption that all members of a population or species are homozygous for 'wild type' alleles at virtually all loci (except a small proportion of polymorphic or mutant genes, which impose a 'genetic load' on their carriers) has been shown by electrophoresis (first of enzymes and proteins, now of DNA itself) to be completely wrong; virtually all individuals are heterozygous for a large number of loci, and this heterozygosity does not produce the debilitating genetic load expected by classical theory (Clarke 1969; Berry 1979; Thornhill 1993). The result has been a recognition that the ties between genetics and ecology need strengthening: genetic determinism has to be modified by ecological understanding while the infuriating prolixity of ecological results can be simplified by a better appreciation of genetic influences (Berry & Bradshaw 1992).

Conservation genetics as ecology

The early development of general rules for conservation genetics involved extrapolating the consequences and dangers of small population sizes and the concept of a 'minimum viable population' (MVP) (later divided into 'genetical' and 'ecological' minima (Gilpin & Soulé 1986)). The simple assumption that population number (with refinements such as sex ratio, proportion of reproducing individuals, etc.) is the key factor for long-term population survival persists (Lande 1988), and has become a standard part of conservation genetics teaching (see, for example Chapter 11 in Primack (1993)). However, the MVP concept is not as straightforward as might at first appear (Soulé & Mills 1992) because:

1. the original estimates of MVP were in the range of 25–50 (MacArthur & Wilson 1967);

2. genetic criteria, agreed at the first international meeting on the application of conservation genetics to wild species in La Jolla, California, in 1978, proposed the so-called 50/500 rule for MVPs, for short-term and long-term programmes respectively (Soulé & Wilcox 1980);

3. then came MVP hyperinflation with recommended values of 10^4 to 10^6 based on simulations of the dynamics of single populations (Belovsky 1987; Goodman 1987), leading in turn to debate about SLOSS (the relative benefits of Single Large Or Several Small populations).

Where does this leave us? In effect MVP inflation has reduced its credibility, and debates about MVP now tend to be carried out in terms of metapopulations, which introduce more biological reality into the discussion than the earlier calculations (Gilpin & Hanski 1991; Lande 1995). I believe we must extend this conflation (or integration) of demography, genetics and ecology; this will surely be to the benefit of ecology *sensu stricto*, never mind its applied offshoot, conservation biology (Berry 1989a).

The genetic constitution of any species is determined by the sum of past environments experienced by the species; to that extent species are prisoners of their evolutionary history. This means that organisms are restricted in their response to any influence, whether biotic or abiotic; put more conventionally, organisms have definable niches. The interactions between genetic limits, possible responses, and environmental heterogeneity and instability have been usefully combined by Southwood (1988). He listed the problem areas in the life of any organism, describing how each results in 'combinations of tactics determined by the potential of the genome. . . for which selection can act. Each of these involves a different amount of risk (i.e. a 'trade off') between alternative courses of action; each of them involves investment in a trait' (Figure 3).

Wallace (1968, 1975) has distinguished between 'hard' and 'soft' selection in a way which complements Southwood's habitat templet. Wallace began from the unsatisfactoriness of the genetic load concept, pointing out the difference between hard selection which acts on alleles that produce their effects in all conditions, and soft selection which involves varying survival (or death) of individuals as conditions change; soft selection is both frequency- and density-dependent, and is likely to vary with other population parameters.

Implicit in this model are two topics which have not had the impact they should have had.

Genetic architecture

In my 1971 paper, I pointed out that genetic architecture produced by the linkage of genes on chromosomes and by the need to maintain biochemically functional mechanisms meant that both developmentally necessary gene combinations and 'super-genes' will be protected; consequently it must be a significant factor in retarding genetic erosion. This point has been significantly neglected since, and none of the contributions in this volume deal with this effect. Virtually all discussions on population variability have been conducted on the straight-forward probabilistic axioms of 'bean-bag genetics' (Haldane 1964). The most likely reason for this has been lack of strong evidence for gene associations in wild populations, apart from a limited number of special cases of tightly linked groups of genes (such as the mammalian histocompatibility complex, incompatibility controls in flowering plants, mimicry patterns in butterflies, colour and shell banding in *Cepaea* snails) (e.g. Barton & Charlesworth 1984). However, failure to find evidence does not mean that genetic architecture is unimportant.

There has been recent interest in testing Mayr's (1954) hypothesis of 'genetic revolutions' (Berry 1996), but this is only a particular case of genetic architecture. As has been the situation with neglecting the evidence for strong selection when attention concentrated on neutralism, so memories of the early work of Mather (1943, 1974), Clausen & Hiesey (1958), Thoday (1961), and others have been displaced by more recent observations with allozyme associations. We must not forget that species are determined by gene associations rather than genes *per se*, and that theoretical considerations indicate that complex traits are likely to be controlled by many loci, selection favouring the formation of 'balanced' chromosomes with positive and negative alleles intermingled. Experimental results (mainly from selection experiments with fruit flies *Drosophila*) confirm this expectation (Bodmer & Parsons 1962). For example the genes which control aggressive behaviour in the house mouse are distributed throughout the genome (Berry 1989b). About one in six of the 1300 identified gene loci in mice can be regarded as affecting behaviour, and they are found in 19 out of the 20 chromosomes in the species.

This neglect of genetic architecture may be changing. Templeton (1986) has discussed the topic in the context of conservation genetics, albeit as it affects outbreeding depression. In a general review of genetics and conservation, Brakefield (1991) drew attention to the

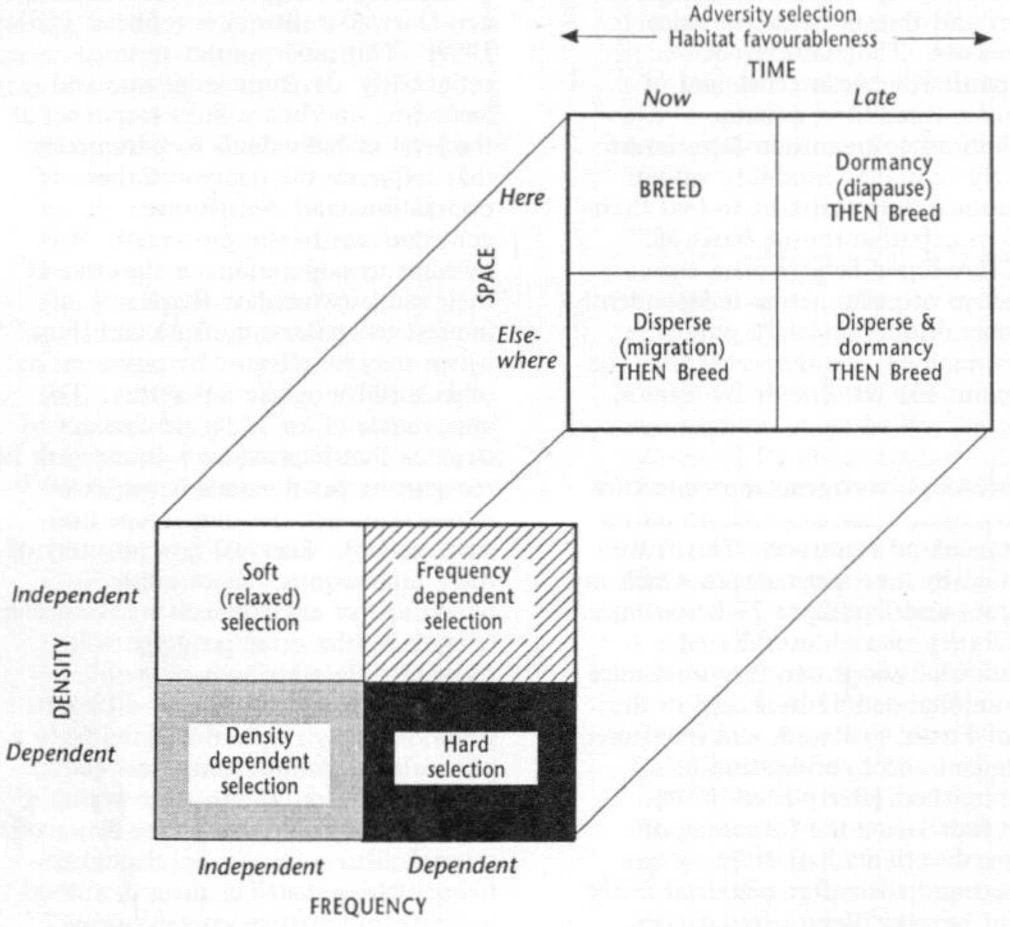

Figure 3. Wallace's (1975) classification of selection complements Southwood's (1988) habitat templet, putting the adaptive adjustability of genetical constitution alongside environmental heterogeneity and introducing the variable stresses experienced by organisms. The links between the two models are not causal: although allele frequencies are commonly regarded as changing with time and in space, frequency may change in space (clinally) and density with time (through recruitment and/or mortality), i.e. selection modes interact with each other and with the environment (from Berry 1992).

work of E.H. Bryant, who showed that despite repeated genetical bottlenecks in experimental populations of house flies *Musca domestica*, substantial variation for evolutionary adaptation remained (Bryant, Meffert & McCommas 1990). Brakefield concluded, 'The results of Bryant's experiments on houseflies are of particular importance to conservation biologists in indicating that populations which have suffered bottlenecks in size, even extreme ones, may retain substantial potential for evolutionary adaptation. Interactions between genes are as yet comparatively poorly understood. The precise mechanisms involved in the phenomena described by Bryant are unclear, as are the relationships between the actual genetic systems and the statistical variance components. The existence of coadaptation between genes and of forms of non-additive genetic contributions to quantitative variation are likely to make variability within populations more resistant to loss than would be expected on the basis of theory developed largely from the perspective of genes acting independently from one another and in a purely additive manner (see Berry 1983)'. The same point has been made by Carson (1990).

The problem about genetic architecture is that we know far too little about its importance and resilience. This is well illustrated by an experiment in which my colleagues and I released 77 house mice from Orkney into an established population of about two thousand mice on an uninhabited 57 ha island in the Firth of Forth, Scotland, and monitored their spread and hybridisation using genetic markers (Berry *et al.* 1990). Within four years, the frequency of introduced variants had more or less stabilised and thereafter remained fairly constant but at different frequencies from those in the receiving population, the ancestors of the introduced animals, and the introduced animals themselves: after a period of rapid change following the introduction, all the new alleles were apparently stabilised at new frequencies by forces undetectable by the techniques available to us, but presumably related to intragenomic associations, i.e. to genetic architecture.

Stress

The second neglected topic is stress; like genetic architecture, this is also not considered at all by other contributions in this volume. Probably the reason for this is a general wariness about the concept, induced by arguments over its correct use (Calow & Berry 1989). A useful working definition for ecologists is that stress 'is any environmental influence that impairs the structure and functioning of organisms such that their neo-Darwinian fitness is reduced' (Calow 1989). This incorporates survival probability, developmental rate and fecundity, and hence links responses at the level of individuals to parameters that influence the density of their populations and their future contributions to the gene pool. It is relevant to populations at the edge of their range (which are frequently of interest to conservationists) and those which may be affected by pollution or other anthropogenic influences. The importance of an adequate concept of stress is that it provides a framework for recognising the dynamic complex of interactions between phenotype and environment. Survival is a property of these interactions, not of either phenotype or environment by themselves (Figure 3); the same genotype will respond differently to a range of conditions through life, and different genotypes may respond differently to a particular environmental stress (Berry, Bonner & Peters 1979). For example, Sikes (1968) found a high incidence of arterial disease in African elephants living at high densities or in disturbed habitats, but virtually none in low-density populations in their natural habitat. Clearly harsh environmental pressures cause problems for living beings, but to evaluate these problems, we need to study organism-environment interactions (or levels of stress) and not merely extrapolate from a knowledge of either genetics or environment.

Implications

Ecology is a synthesis of disciplines, concerned with explaining why is what where. Evolutionary biology is even more obviously a synthesis of different subjects, and it is salutary to recall the repeated trauma it has suffered by allowing its components to fall apart. As conservation geneticists drawing upon both ecology and evolutionary genetics, we need to be particularly aware of the dangers of distortion through over-simplistic interpretation, and the consequent hazards as we attempt to apply our results as conservation managers. There are five areas of importance.

1. We need four kinds of data. First, almost all biological systems are special cases, so we must collect information on many food webs, breeding systems, ecosystem stability and inertia, as a deterrent to generalising from inadequate information. Secondly, data on a long-term basis: Gray (1989) has reviewed the effect of pollution on marine benthic macrofauna, using abundance-biomass comparisons (ABC curves) to show a characteristic sequence of response involving a rapid increase of biomass, followed by a fall in the number of species, then dominance by a few, small-sized opportunist forms, until both the number of species and biomass decreases. He points out that these changes are paralleled by the normal situation in the intertidal zone; they are also comparable to those in colonising and successional stages generally. Thirdly, data are required on life-history variation and its causes, since this provides a tool for investigating its significance; it can (and should be) an important corrective to the all-too-common assumption that life-tables are an approach to a definitive description of a species' biology. And finally, tropical ecosystems are species-rich in comparison with temperate ones. Too little is known about the status of tropical species, particularly whether temperate morphs are equivalent to tropical species (Hubbell & Foster 1986).

2. The effect of stress on ecosystems can often by detected by changes in energetics, community structure or system characteristics (such as openness, disease incidence, mutualism, etc.) (Rapport 1989; Jones & Lawton 1995). These effects in turn frequently reflect life history changes for individual species or populations. As we accumulate data, we will be better able to predict and hence manage biological systems (Spotila *et al.* 1989).

3. There is a persisting habit among conservationists to assume that change is necessarily detrimental and that there exists an innate 'balance of nature' (McIntosh 1985; Botkin 1990). Both these assumptions must be resisted; the notion of an unchanging world owes more to Greek philosophy than modern biology (Egerton 1973). Anthropogenic changes in the natural world are now proceeding at a frighteningly dangerous rate, but it is no help to aim for the *status quo*. Although it is wrong wantonly to exterminate species, we have to accept that some species are more 'important' than others (in terms of their interactions, biomass, distribution, etc.), and that habitat conservation is more important than species conservation. And heretical though it may seem, DNA techniques are likely to help us to maintain gene combinations more easily in culture than to preserve whole species.

4. Linked to the notion of a 'balance of nature' is that of a 'saturated habitat'. In practice, virtually any habitat is susceptible to invasion, although the characteristics of invaded habitats or of the invaders themselves have so far proved impossible to define. The common assumptions of ecologists and geographers about such systems are too static to explain observed invasions, and depend too much on equilibrium-type theories (Hengeveld 1989).

5. We need to be much clearer than hitherto about our motives for conservation. Much of the rhetoric about the probable value of undescribed species for mankind is unconvincing. We can almost certainly preserve most genetic variety *sensu stricto* in gene and embryo banks if that is all we want to do. But we want – and need – to do more (Given 1994). Norton (1987) has argued for the importance of species conservation on 'weak anthropocentric grounds', that we are transformed by our contacts with natural variety in a powerful and effectively humanising way; the instrumental value of living animals and plants (i.e. 'strong' anthropocentricism) is relatively unimportant in comparison.

This brings us full circle, and back to my designation by Frankel & Soulé (1981) as a 'phyletic optimist' for believing that variation loss may not be as common or catastrophic as is sometimes claimed. There can be little doubt that human activities threaten our continued existence, but I believe that our efficiency in eroding the world's overall genetic resources is probably not too great. We may lose species, but that does not necessarily mean that the genetic variation carried by them is also lost; many gene combination and chromosomal segments are common to different species. The real danger is assuming that genetic *processes* are therefore unimportant. We may not know the links between gene, species and ecosystem diversity in any particular situation, but we know enough to recognise that the different levels are interdependent. This means that our *attitude* to our environment is crucial; species conservation is both symbolic and symptomatic of our commitment to an environmental stewardship on which the biological future and quality of life of the planet depends (Berry 1990).

John Avise concluded a major review of the impact of molecular biology on natural history and evolution by pointing out that molecular perspectives (he could have written genetical knowledge) "do not supplant traditional approaches in the context. . . but rather enrich our understanding of life. Herein lies the greatest value of molecular methods in conservation biology or elsewhere" (Avise 1994, p. 398). Introducing the (American) National Forum on Biodiversity in 1986, E.O. Wilson (1988) concluded "In the end, I suspect it will all come down to a question of ethics – how we value the natural worlds in which we evolved and now, increasingly, how we regard our status as individuals. . . The drive towards perpetual expansion – or personal freedom – is basic to the human spirit. But to sustain it we need the most delicate, knowing stewardship of living world that can be devised." Conservation genetics involves moral choice as well as the best support from its parent sciences.

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What genetics does the conservation officer need to know?

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Spencer, J.W. 1997. What genetics does the conservation officer need to know? *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, 33–38. Peterborough, JNCC.

Conservation genetics is concerned with changes in genetic variation over long periods of time and conservation initiatives have to allow for this in their planning. Much of the importance of the present pattern of genetic variation in our native wildlife is not self-evident and its meaning is not always intuitively obvious; it becomes more apparent through detailed study and observation and has to be interpreted through an understanding of population biology and genetics. As nature conservation becomes more proactive and interventionist, in the face of both increased threats and heightened opportunity, professional conservation officers need to acquire a more comprehensive understanding of population processes and the genetics of the organisms they are responsible for.

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Introduction

Nature conservation is predominantly a practical activity and as such it uses the natural sciences, amongst other disciplines, to inform its practitioners about the likely effectiveness of their decisions. It is not just about habitats and species; it is also about conserving the existing meaning of places (that elusive combination of facets that makes up the *genius locus* of a site), along with present-day opportunities for study and future opportunities for enjoyment or exploitation. As this has become more widely appreciated, the interests encompassed by those involved in nature conservation have grown, and now include geology, soils, long-term studies of natural processes, and the economics and politics of land use, alongside the now more traditional areas of vegetation studies, habitat management, and the conservation of rare plants and animals. Genetic studies have extended this list yet again, and the maintenance of native genetic variation in British plants and animals, and the protection of places of note for its study, have become legitimate conservation goals.

In the course of their work, conservation officers deal with a very wide range of priorities in an increasingly complex world. Many of the priorities are influenced by other institutions driven by other objectives. The officers of the statutory nature conservation agencies are looked to for advice and support in all manner of situations, and in recent years these have included an increasing demand for advice on population biology, ecological genetics, and their implications for nature conservation. As genetics has become more frequently used to inform the decisions of conservation practitioners, they have increasingly come to appreciate the importance of the subject in their activities. However, there is often a profound difference in perception between ecologists and geneticists: "Ecologists often treat all members of the same species as having identical properties", whereas "geneticists have little understanding of the lifestyle of organisms and hence how natural selection and population processes work" (Raybould 1993).

Uncertainty over the role and importance of genetics in nature conservation remains widespread in the conservation community, and until recently the conservation of genetic variation within species was not regarded as a high priority. The urgency of habitat conservation has always appeared to be so much more pressing. Now, however, there is an increasing acceptance of the importance of genetics, with a growing number of projects underway which fully acknowledge the need to be informed of the genetic principles underpinning them. This paper is aimed at describing the gap that presently needs to be bridged between those involved in research into genetics and those involved with the practice of nature conservation.

The problem of time

Few people have any real appreciation of the time-scales involved in any serious, long-term nature conservation initiatives. Nor do they appreciate the human implications of the passing of such long expanses of time. This presents some major obstacles to comprehending the difficulties that will be encountered by such projects, and yet which need to be anticipated in their design from the outset. Nature conservation has to take the long view, aiming to achieve objectives that will last for a very long period of time. Soulé *et al.* (1986), in their discussion on the Millennium Ark, are of the view that conservation effort will have to make provision for the next 500 to 1000 years to provide for the difficult times ahead, termed by them the "demographic winter". Society might then be able to look forward to a time when land and opportunity are available to reconstruct or enrich quasi-natural ecosytems on a meaningful scale. Conservation projects demand long maturation times, often very much longer than the working lifetimes of the individuals involved. In projects involved with the conservation of genetic resources it is excessively longer. (In this respect they share and suffer from similar problems to woodland creation projects; see Spencer (1995).) Any project that is to make a lasting contribution has to be robustly planned, given the fast turnover of fashions in nature conservation, land use and scientific research. The open-ended time-scales demand initiatives that must anticipate and accommodate changes completely unforeseen by the project planners, and be designed with an appropriate robustness of purpose. Can society reasonably set out to pursue a project that is not expected to come to fruition for centuries to come? The genetic models that inform the conservation debate insist that we look to the long term and design our projects to be both robust and lasting if they are to be anything other than of transitory worth.

Genetics and the conservation of meaning

Much of the value of our rarer plants and animals lies not in themselves, but in what they can tell us. Their ecologies, past and present distributions, and their behaviours under different circumstances all tell us a great deal about past environments, climates, changes in land use, human activities and the response of the natural world to these complex and ever-changing conditions. Genetical studies add another dimension to this list, illuminating the ways in which the genetic make-up of populations of plants and animals respond to change. Such studies are important in the lessons they have to offer about the consequences of future man-made change. They also add to our understanding of the meaning of the British landscape, a landscape of particular importance in the study of man's impact on the natural world. In Britain, the consequences of our activities have been considerable. There is no doubt that they have taken place over very long stretches of time and greatly reduced the biological richness of the landscape in the process. Nevertheless our historical records, the quality of our archaeological science and the detailed knowledge we have of the ecology, distribution and history of our fauna and flora are exceptional on a worldwide scale. In consequence, the conservation of the ecological, historical and evolutionary meaning of our natural heritage is not just a question of enjoyment and appreciation, it is also a question of international scientific importance.

Recent studies on the population genetics and genetical systems of rare plants and animals, such as the studies on relict populations of *Draba aizoides* and *Mibora minima* in Wales (see Kay & John this volume) and the behaviour of genes in an expanding population of reintroduced red kites *Milvus milvus* (see Cordero *et al.* this volume) add a genetical perspective to this rich documentation. With time this will no doubt expand to cover many more organisms and situations. Such studies are increasingly adding to our understanding of the response of our native organisms to their environment.

The conservation of meaning is a difficult concept to promote or to adopt. Most meaning lies hidden, waiting to be discovered and interpreted. Caution and thoughtful appreciation of the potential of sites for informative investigation, and the application of the precautionary principle when planning introductions, translocations and other population manipulations, would go a long way to ensuring a minimal loss of valuable meaning and important information from any site or situation. Conserving these opportunities for future study is a key, if presently undervalued, component of nature conservation (Kay 1993).

Genetics and biological conservation

In recent years nature conservation has been able to take up the offensive, and is now attempting to recover some of the ground lost over the last 100 years. Projects such as the reintroduction of the red kite to lowland England (Evans & Pienkowski 1991), and the many other planned species restorations and re-creations of semi-natural habitats, have begun to take their place alongside the more traditional work of conserving and managing the surviving tracts of semi-natural habitat and sites for rare and endangered wildlife. With this growing emphasis on restoration, re-creation, recovery and re-instatement, there comes a far greater need to understand and apply the principles of genetics and population biology. There is simply far greater scope for initiating the loss of variation, meaning and information in interventionist projects than there is in simply hanging on to what you have!

Research work and case studies are very important. The results of genetic research are not always intuitively obvious. The two population clusters of *Draba aizoides* on the Glamorgan coast illustrate this very well. They lie very close to one another and are believed to be late glacial relicts. Research into their genetics supports this view, but it also demonstrates that, if derived from a common source, they have nevertheless been isolated from one another for many thousands of years (John 1992). Their very proximity to one another might lead one intuitively to conclude that they are part of the same contemporary population; the genetic evidence points to a quite different conclusion. The structure and variation within these two small populations is full of meaning that needs to be conserved. Such information is not self-evident from any amount of casual or more orthodox taxonomic examination, and an understanding of the genetics of the situation has considerable implications for the conservation of this species in Wales.

Similar researches have revealed some surprising details of the genetic relationships between populations of brown bears in Europe. The Pyrenean brown bear is critically endangered and presently the subject of considerable debate. It is not clear what conservation measures are required to save it from extinction and many of the options have scope for greatly changing the present genetical structure of the surviving relict population. Recent research (Kohn *et al.* 1995) has shown that the Spanish bears are more closely related to bears in Scandinavia than to other bear populations in southern and eastern Europe. Bears from southern Scandinavia and western Europe, including the Pyrenees, are part of an extensive 'western lineage' that differs in its mitochondrial DNA from the 'eastern lineage' bears of Russia, Bulgaria and parts of Romania. Both lineages are further subdivided into smaller, locally distinct, populations. The two lineages are regarded as very distinctive genetically, with the origins of this variation rooted in their pre- and post-glacial distribution and dispersal. Conservation programmes aimed at maintaining bears in these countries have to be aware of these differences and act accordingly if the full range of genetic variation is to be conserved and its geographical meaning maintained. Any introduction of bears from outside the region may compromise the genetics of the local population, yet there are almost certainly not enough bears to survive through the next 50 years or so. Genetical studies extend our understanding of the nature of the problem and may help us to resolve the challenges of setting priorities in such situations.

There may also be important adaptive reasons for maintaining the distinctive patterns of genetic variation found in geographically distinct populations. Experience with the deliberate mixing of races of deer in North America and ibex in Europe has exposed native populations of ungulates to the serious disruptive effects of breaking up co-adapted gene complexes influencing reproductive physiology and behaviour (Galindo-Leal & Weber 1994). The consequences of thoughtless mixing of distinctive races, long-adapted to particular climatic regimes or habitats across their ranges, can be both serious and unpredictable.

The cases above illustrate the non-intuitive nature of many issues involving genetics and population biology. To guide conservation officers across the neglected discipline of population genetics, there is a need for a much closer understanding of the underlying principles, and a wider range of carefully targeted research work, case histories, precedents and practical examples to draw on. Appraisal and caution is needed before action is taken, and clear objectives need to be established before the practical work begins.

What the conservation officer needs to know

Much has been achieved by the conservation movement over the last two or three decades and, until recently, with limited financial resources. Conservation officers are backed up by an array of advice, support, research and literature on issues ranging from hydrology to planning law. However, neglect of genetics and its implications are widespread. Most staff have only a rudimentary level of training in genetics, and the situation is exacerbated by the science of genetics becoming more complex and technical. There is, though, growing recognition of the importance of genetics and the need for conservation decisions to be informed by an understanding of its implications.

Conservationists already make use of genetic case studies in effectively arguing the conservation case. The first example I came across was a persuasive conservation officer talking a Suffolk farmer out of grubbing up an ancient wood on the grounds that it was one of the few sites in East Anglia for the rare perennial wood barley *Hordelymus europeaus*. The farmer could see the sense of conserving the potential for a perennial barley to be used in future breeding programmes even if he could not see the point of keeping an ancient wood! Other more widespread species offer great potential for making similar points (Soutar & Spencer 1991; Hancock & Luby 1993). The rationale for conserving rare and often unimpressive lichens, fungi and small invertebrates frequently rests on a similarly perceived potential for human use. The utilitarian case for conserving genetic variation may often be overstated; however, conservation officers can only pursue such cases with conviction when they are equipped with strong arguments and eloquent case studies, backed up by a credible degree of understanding.

Conservation officers need advice and support in situations involving small, isolated and relict populations, rare species, widespread but variable species, captive-breeding projects and re-introduction programmes. They need help in developing cogent and coherent arguments to inform their negotiations. At a higher level, there is also a need for local studies to be complemented by landscape-scale studies on the nature of variation to inform national conservation activities and strategies.

In summary the conservation officer needs:

(a) an appreciation that biodiversity extends below the level of the species;
(b) an understanding of how patterns of variation can vary enormously between species with different reproductive systems and population histories;
(c) a recognition of the importance of levels and patterns of intraspecific variation both in an evolutionary context and as a source of historical information;
(d) a sound appreciation of the genetic implications of management intervention.
(e) advice on priority taxa for conservation attention;

With this understanding to hand, the officer will be better placed to act effectively to conserve both the levels *and* patterns of genetic variation within species.

The setting of priorities is a key issue. Resources for describing and conserving genetic variation are always likely to be limited. Small populations of edge-of-range species with high public profiles (notably rare orchids) corner large slices of limited resources. Expensive mitigation measures are often wasteful of resources that could be more profitably spent elsewhere by society, whether on nature conservation or in other pressing areas of need. The recent demand to remove 80,000 great crested newts *Triturus cristatus* from a series of pits destined for development is a good example of where an appreciation of population genetics might have led to a more cost-effective approach. Most population models would suggest that a few hundreds of newts would be sufficient to retain the bulk of the native variation (i.e. a bucketful or two!), and it is clear that the fate of most of the newts will depend on the carrying capacity of their new home. To attempt to remove all the newts to newly created ponds is inefficient of both effort and expense. With limited resources of time, space and money available for nature conservation, population biology and genetics are going to become increasingly important tools for informing and guiding us towards hard but effective decisions.

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Section 2 – Conservation implications of genetic survey data

The five papers in this section comprise a series of case studies of rare or declining species. They include discussion of the genetic techniques used and the implications they carry for conservation management.

Quentin Kay and Rosemary John give several recent examples from the plant world of how genetic survey data could, and should, influence practical conservation management of rare or declining species. They affirm that knowledge of the extent and distribution of genetic variation is of central importance in the planning and execution of conservation strategies. They warn, however, of the dangers implicit in inappropriate generalisation between species and in extrapolating from theoretical situations to those on the ground.

The conservation genetics of rare British grasses are considered by Alan Gray. He stresses the extent to which genetic principles, based on an understanding of the genetic system and genecological circumstance, can inform species conservation strategies. He argues that unrealistic ideals, such as the wish to conserve each and every population, should be replaced by realistic and achievable strategies for the quantifiable conservation of genetic variation.

The genetic variation in a rare Spanish endemic flowering plant was investigated by Honor Prentice and Stefan Andersson. As with many species, they conclude that although habitat destruction is the most proximal factor threatening the species, an understanding of its genetic variation by conservationists is crucial. Not only is genetic variation important, but we must be careful where we look for it. In their study, variation between subpopulations is as important as variation between populations, and this has important implications for practical management.

Richard Ennos and his colleagues use a case study of the Scottish primrose to illustrate some of the potential pitfalls in the headlong rush to embrace conservation genetics. Whilst it is clear that genetic variation is crucially important, molecular techniques such as isoenzyme electrophoresis may not, in every case, give an appropriate estimate of genetic variation and the adaptive potential of a population. In some instances, traditional methods of quantitative genetics may be more effective at answering the questions posed in long-term management strategies.

Often, however, molecular genetic techniques can provide valuable information for conservationists. Andrew Pullin and his co-authors consider that, for some insects, reduced gene flow and extreme isolation of populations suggest that site-based conservation strategies are likely to be ineffective for maintaining genetic variation and that wider countryside initiatives may point the way forward. Pullin *et al.* also use genetic data to resolve the taxonomy of British brown argus butterflies and review the conservation priorities associated with its different forms.

This section shows that no single technique will provide a panacea for everything that the practical conservationist requires. Nevertheless, if we fail to undertake, or ignore the results of, genetic survey then we risk making grave errors (such as the introduction of inappropriate genotypes) that cannot subsequently be corrected.

Patterns of variation in relation to the conservation of rare and declining plant species

Quentin Kay and Rosemary John

Kay, Q., & John, R. 1997. Patterns of variation in relation to the conservation of rare and declining plant species. *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, 41–55. Peterborough, JNCC.

Patterns of genetic variation differ widely in different plant species. They are affected by the history of the species and of its component populations, and by its population structures, breeding system, life-form and reproductive biology. They are of particular and sometimes crucial importance in rare and declining species. In the British and Irish floras, very few full species are endemic or absolutely rare on a world scale, but the isolated populations of our rare species often have patterns of variation which are of great interest. In old-rare plant species, they may mirror the history of the populations and show their origins and relationships, sometimes in considerable detail. The examples of *Draba aizoides*, *Mibora minima* and *Ononis reclinata* are described. In newly-rare and declining species, they also give an indication of the likely genetic future and prospects for survival of isolated populations; examples include *Carum verticillatum*, *Chamaemelum nobile*, *Cirsium dissectum* and *Vicia orobus*. Knowledge of these patterns of genetic variation is of central importance in conservation of rare and declining plant species, especially when active on-site or off-site 'recovery' measures involve, or envisage, breeding programmes and population reinforcement or the establishment of plants in new sites.

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Introduction

During the Pleistocene period, the flora of Britain and Ireland was subjected to great disturbance, with repeated southward and northward migrations during a succession of glacial and interglacial phases. Apart from some arctic-alpine and montane species, the bulk of the present native flora of Britain and Ireland arrived during or since the end of the Devensian glaciation about 12,000–10,000 years ago, especially during the Late-glacial and early Post-glacial periods when sea levels were still low and land bridges remained. Godwin (1975) estimated that at least 67% of the present flora had arrived before the isolation of Britain from continental Europe by the formation of the English Channel about 7000 years ago, although Ireland and many of the islands around the coasts of Britain were isolated at a somewhat earlier stage. The main sources of the new flora were areas of southern and south-western Europe where the climate had remained temperate during the Devensian period.

Although theoretical considerations suggest that significant numbers of endemic full species of plant would have had time and opportunity to evolve in Britain and Ireland during stable glacial or interstadial periods, very few absolutely rare (in world terms) or endemic full species either survived or emerged from the post-Devensian process of climatic and ecological change and migration in Britain and Ireland. *Fumaria occidentalis* and *Primula scotica*, both of which are allopolyploids and likely to be of recent origin, are the best examples of endemic full species, with *Coincya wrightii*, a self-incompatible diploid (Harberd 1972; Kay unpublished), apparently endemic to Lundy Island, probably being the sole example of an outbreeding full species that is both endemic and absolutely rare in Britain. Absolutely rare non-endemic full species are also very few in the British and Irish flora; *Rumex rupestris* may be the best example, among perhaps fewer than five candidates.

On the other hand, regional rarity has flourished in Britain and Ireland, and continues to affect a steadily increasing number of full species (Perring & Farrell 1983; Curtis & McGough 1988). The ranges of many formerly widespread arctic-alpine and northern species were fragmented as climatic amelioration drove them into cliff and montane refuges, reducing them to local rarity after 12,000–10,000 BP. Similar processes took place later for many widespread open-habitat species of the Late-glacial, which were driven into cliff and coastal refuges as forests spread during the Post-glacial (10,000 BP onwards); for some species dependent on woodland as forests were cleared or disturbed by Neolithic and later farmers (6000 BP onwards); for wetland and bog species as their habitats were drained or destroyed by man (about 2000 BP onwards); for weeds of arable land as better seed-cleaning, changes in agricultural practice and finally synthetic herbicides largely eliminated all but the most persistent and adaptable species (about 100 BP onwards); and most recently for plants of ancient grassland, as reseeding and intensive fertiliser use destroyed this habitat (about 50 BP onwards).

Patterns of genetic differentiation in locally or absolutely rare plants depend on several factors: their breeding system, reproductive biology and life-history; the history, size, structure and degree of isolation of their local populations; natural selection; and perhaps also hybridisation with other taxa. Most of these factors vary much more in plants than in animals. Effects related to breeding system and population structure range from the near-complete absence of genetic variation in apomicts (for example microspecies of *Hieracium*, *Limonium* and *Sorbus*) through the stepped patterns of variation often seen in autogamous species with poor dispersal (for example *Ononis reclinata*) to the interrupted clines and extensive stochastic variation of partial or complete outbreeders with small isolated populations (for example *Mibora minima*). A few locally rare or sparsely distributed outbreeding species with exceptionally good dispersal (for example some orchids with dust-like seeds, and some waterside plants dispersed by waterfowl) may maintain overall gene-flow and more normal patterns of continuous ecoclinal variation and local adaptation, but most outbreeding rare species are likely to show patterns of variation which are disjunct, partly stochastic, and often highly diverse.

In plant conservation and recovery programmes, knowledge of the breeding system and the patterns of genetic variation of the species that are involved is a basic and essential requirement (Barrett & Kohn 1991; Falk & Holsinger 1991; Karron 1991) and should be regarded as a priority. Because these fundamentally important biological factors can be so different in plants, concepts of minimum viable population size, sample size and breeding strategies derived from studies of animal populations are often entirely inappropriate in plant conservation, and may lead to serious errors (Templeton 1991; Lesica & Allendorf 1992; Schemske *et al.* 1994). Isozyme studies now provide a rapid and practicable means for assessing patterns of variation in and among populations of many plant species (Schaal, Leverich & Rogstad 1991; Prentice 1992). Knowledge of the breeding system, reproductive biology and pattern of genetic differentiation of a rare plant taxon is essential not only for a recovery programme (e.g. Whitten 1990) to have a real chance of success, but also to avoid endangering existing populations and losing, contaminating or distorting the existing pattern of variation. This pattern of variation is often in itself an evolutionary and historical document of great potential value, not only in absolutely rare species, but also, and perhaps especially, in locally rare species, including *Draba aizoides*, *Ononis reclinata* and *Mibora minima* in Britain and western Europe (John & Kay 1989, 1990; John 1992). In threatened or declining species like *Cirsium dissectum*, *Hypericum linariifolium*, *Luronium natans* and *Vicia orobus* (Kay & John 1994, 1995) knowledge of these factors can also document genetic impoverishment and remaining levels of gene-flow and heterozygosity.

Patterns of variation in some rare British species

Yellow whitlowgrass *Draba aizoides*, an attractive, saxifrage-like crucifer, occurs in Continental Europe on montane cliffs, mainly in the Pyrenees, Alps and Carpathians. In Britain it grows only along about 17 km of maritime cliffs in the Gower Peninsula in southern Wales. These cliffs are a well-known refuge site for several scarce or rare species of open habitats, including Late-glacial survivors like *Helianthemum canum*. *Draba aizoides* is a self-fertile, short-lived perennial with a high reproductive capacity; its British populations are firmly established in a specialised habitat and do not appear to be threatened, nor to have changed in their overall distribution since their discovery in 1795 (Kay & Harrison 1970). The history and origin of the British populations are uncertain. It has been suggested that it might be an escape from cultivation in Britain (e.g. Schulz 1927; Clapham, Tutin & Warburg 1962); these suggestions were speculative, but were supported by the late discovery of the plant in Britain, its unusual ecological situation there, and the wide disjunction of its British populations from its nearest Continental sites. There is a small population of uncertain status near Namur in Belgium, but the nearest large populations are in central France and in the Swabian and Franconian Jura in Germany, about 850–900 km from the British sites.

Studies of isozyme variation in *D. aizoides* in Britain, and in 17 Continental European populations (John & Kay 1990; John 1992), showed high levels of variation, with a total of 48 alleles at 12 polymorphic loci (Table 1). Coefficients of outbreeding were fairly high in Britain, ranging from 0.6 to 0.8, but were lower in some Continental populations where the range was from 0.2 to 0.8. Calculations of the genetic interrelationships between populations based on the occurrence and frequency of alleles show that the British populations have no affinities with any particular Continental population among those that were studied, and are in fact markedly separated from the Continental populations in terms of genetic distance. In addition, despite their limited extent, the British populations were found to show considerable genetic differentiation among subpopulations, with differences between the eastern and western groups (which are separated by a gap of 8 km) and clear geographical trends from west to east; the less common alleles are usually confined to small geographical areas (Figure 1). They are however genetically somewhat depleted in comparison with Continental populations, with a total of only 20 alleles and a maximum of three at a single locus in Britain. The genetic evidence thus suggests that the British populations have been isolated from Continental populations for a considerable period of time, probably many thousands of years; that they have had their present distribution in Britain for a very long period; and that at some time prior to this there was a

Table 1. Isozyme variation in some scarce and declining plant species in Britain and Ireland. Data from John (1992) and Kay & John (1994, 1995). Genetic distances after Nei (1972). Numbers of monomorphic loci found are shown in brackets in column 4 only for species in which fewer than 6 polymorphic loci were found.
Abbreviations: n/a, not appropriate (see text and Figure 4); in.d., insufficient data.

	Populations			**Variation**		
	Number of populations sampled	Mean sample size	Range (km) of sampling	Number of poly. loci	Maximum genetic distance	Mean % poly. loci
Draba aizodes (Europe)	17	21.4	900	12	0.692	54.7
D. aizodes (Wales)	22	27.3	17	9	0.284	28.4
Mibora minima	31	33.3	680	9	0.372	77.8
Ononis reclinata	15	10.7	2850	1(8)	n/a	0.7
Carum verticillatum	41	30.2	460	7	0.830	77.0
Chamaemelum nobile (All)	26	12.0	420	7	0.548	24.7
C. nobile (Wales)	8	12.5	140	4	0.283	12.5
Cirsium dissectum (Wales)	20	23.0	150	1(7)	0.003	2.1
C. dissectum (Ireland)	5	26.7	300	4(4)	0.548	32.1
Vicia orobus	21	23.7	150	9	0.805	74.7
Carex montana	12	13.1	270	9	1.338	65.7
Cirsium tuberosum (All)	5	18.4	120	6	0.287	37.2
C. tuberosum (Wales)	3	14.0	3	4	0.029	28.6
C. tuberosum (England)	2	25.0	9	5	0.091	50.0
Cytisus scoparius ssp. *maritimus*	9	17.0	580	9	0.289	93.2
Eriophorum gracile	5	13.2	320	1(7)	0.135	2.5
Gentianella uliginosa	2	10.0	10	0(7)	nil	nil
Hypericum linariifolium	14	12.7	430	6	0.566	82.0
Hypericum undulatum	7	19.9	150	6	0.466	40.5
Liparis loeselii	4	2.0	420	0(9)	nil	nil
Luronium natans	17	5.1	440	4(7)	1.120	35.3
Mentha pulegium	10	6.1	2500	9	1.172	88.9
Pilularia globulifera	3	6.3	24	0(14)	nil	nil
Potentilla rupestris	2	6.0	660	3(4)	in.d.	83.3
Salvia pratensis	5	8.0	220	5(4)	0.583	64.0
Stachys alpina	2	5.0	170	2(11)	in.d.	25.0
Wahlenbergia hederacea	11	23.0	500	11	0.670	67.3

severe bottleneck, either because the initial colonising population consisted of a small number of plants, or because of reduction in numbers as a result of environmental factors. A Late-glacial date of arrival in Britain for *D. aizoides* seems possible in view of its association with other Late-glacial refuge species and its absence from arctic-alpine habitats here. As its altitudinal range in Europe extends to more than 3000 m in the Pyrenees and 3400 m in the Alps, an alternative although somewhat less likely possibility is that it could have arrived at an even earlier stage and survived in or near its present sites in Britain during the Devensian glaciation.

Indications for conservation are that the British populations are genetically viable, and that their present pattern of variation should not be altered by interpopulation transfers.

Early sand-grass *Mibora minima* is a tiny (about 1–5 cm at flowering), inconspicuous and very early-flowering (January to mid-March in Britain)

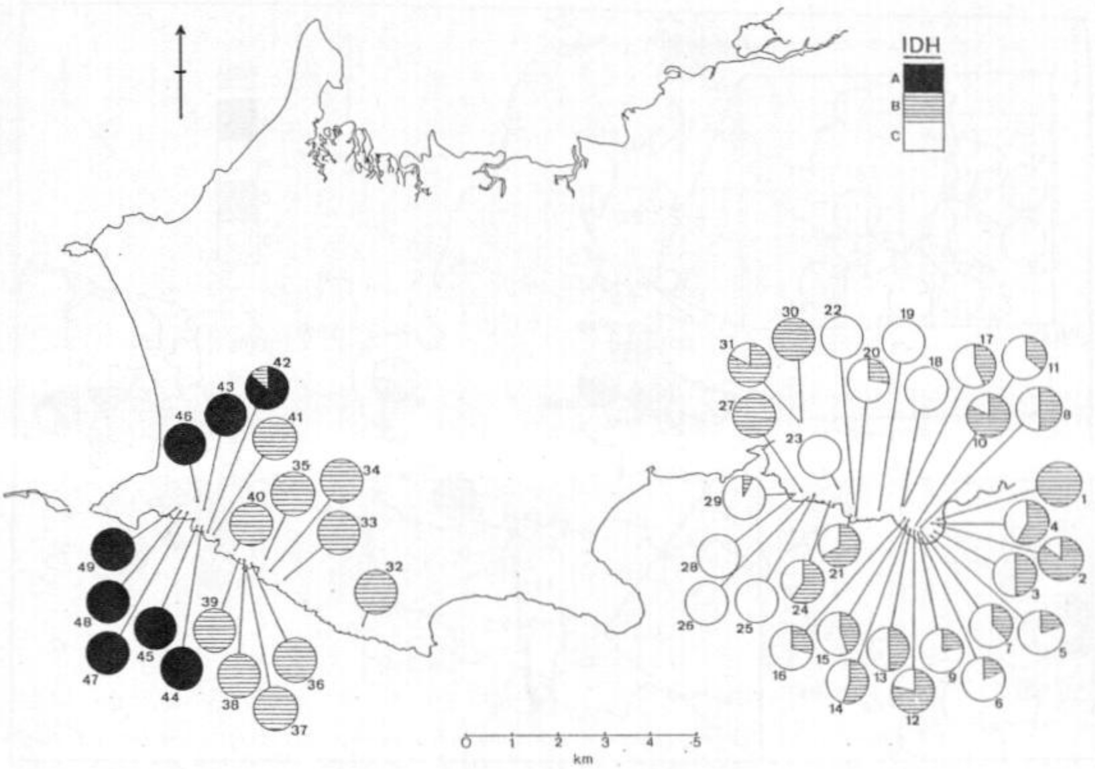

Figure 1 Allele frequencies in *Draba aizoides* populations and subpopulations in the Gower Peninsula; isocitrate dehydrogenase (*IDH*).

annual grass. It is very rare in Britain, where populations that appear to be native are known only on a few coastal dune systems in Anglesey and one in Glamorgan. Established populations that are thought to be introduced also occur on the Scottish coast near Edinburgh, and on Studland Heath in Dorset, with some casual records elsewhere. Its Continental populations are scattered but fairly widespread, mainly in western Europe northwards to Holland, but often inland as well as in coastal sites. In the Channel Islands it is frequent on coastal cliffs. In Britain, the Anglesey populations have been known for many years and have always been regarded as native, but the Glamorgan population (at Whiteford Burrows in Gower, covering about 2 ha of old fixed dunes) was discovered only in 1964 and was of uncertain status. It at first seemed possible that the Whiteford population might be a recent introduction, perhaps from Anglesey. Although the demography and ecology of the Anglesey populations have been quite intensively studied (Pemadasa, Greig-Smith & Lovell 1974; Pemadasa & Lovell 1974a, 1974b, 1974c) nothing was known of the genetic relationships between British populations and those in the Channel Islands and Continental Europe, and the breeding system of the species was uncertain.

Studies of the reproductive biology of *M. minima* and of isozyme variation in 12 populations or subpopulations from Britain, 11 from the Channel Islands and eight from The Netherlands (John & Kay 1989; John 1992) showed that it was wind-pollinated, strongly protogynous and largely outbreeding, with high levels of heterozygosity, despite its diminutive size and annual habit. The general level of variation was high, with 42 alleles being found at nine polymorphic loci (Table 1), for example phosphoglucomutase

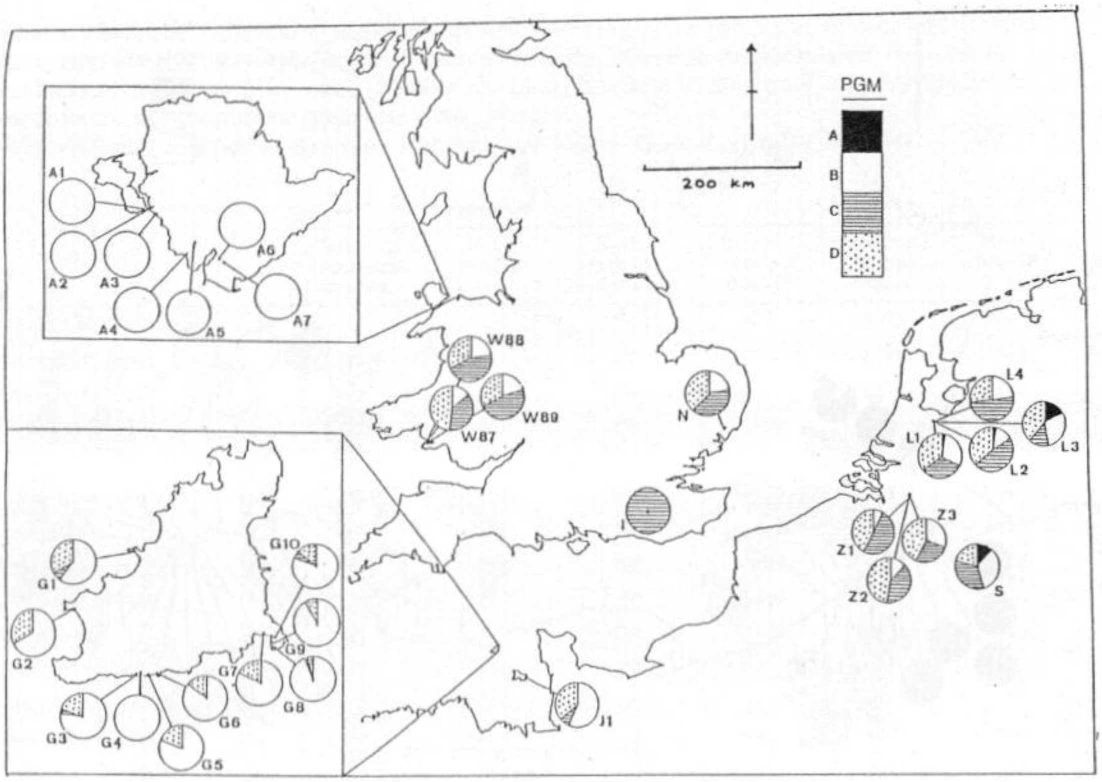

Figure 2 Allele frequencies in *Mibora minima* populations in Britain, the Channel Islands and Holland; phosphoglucomutase (*PGM*).

(Figure 2). Calculations of the genetic distances between populations based on the occurrence and frequency of alleles (Figure 3) show strong regional groupings of populations from the same areas (Anglesey, Guernsey, Holland, Whiteford) but with considerable genetic differentiation between populations where several exist within an area. The Whiteford and Anglesey populations are genetically widely separated; the Whiteford population possesses seven alleles not found in Anglesey, so could not be an introduction from Anglesey. However, all 22 alleles found at Whiteford also occur in a single population at Jerbourg in Guernsey, suggesting the possibility that the Whiteford population might be derived by long-distance dispersal from the Channel Islands or from a related population. Allele frequencies are, however, very different in the two populations. The Guernsey and Netherlands groups, each of which shows interesting and informative patterns of inter- and intra-population variation, are genetically rather distant from each other. Some of the Netherlands populations grow as weeds of plant nurseries, and show genetic affinities to the one naturalised British nursery population that was investigated (Woodbridge in East Suffolk) which thus appears to be derived from Continental stocks and not from any native populations in Britain or the Channel Islands. A single commercially available, deliberately propagated stock from another British nursery (East Grinstead in West Sussex) was also investigated; it is genetically rather distant from all other populations, and, most exceptionally, is completely homozygous at all the nine loci that were tested, probably as a result of severe inbreeding and bottlenecking in cultivation. Of 656 plants tested from other populations, only five were monomorphic across all nine loci.

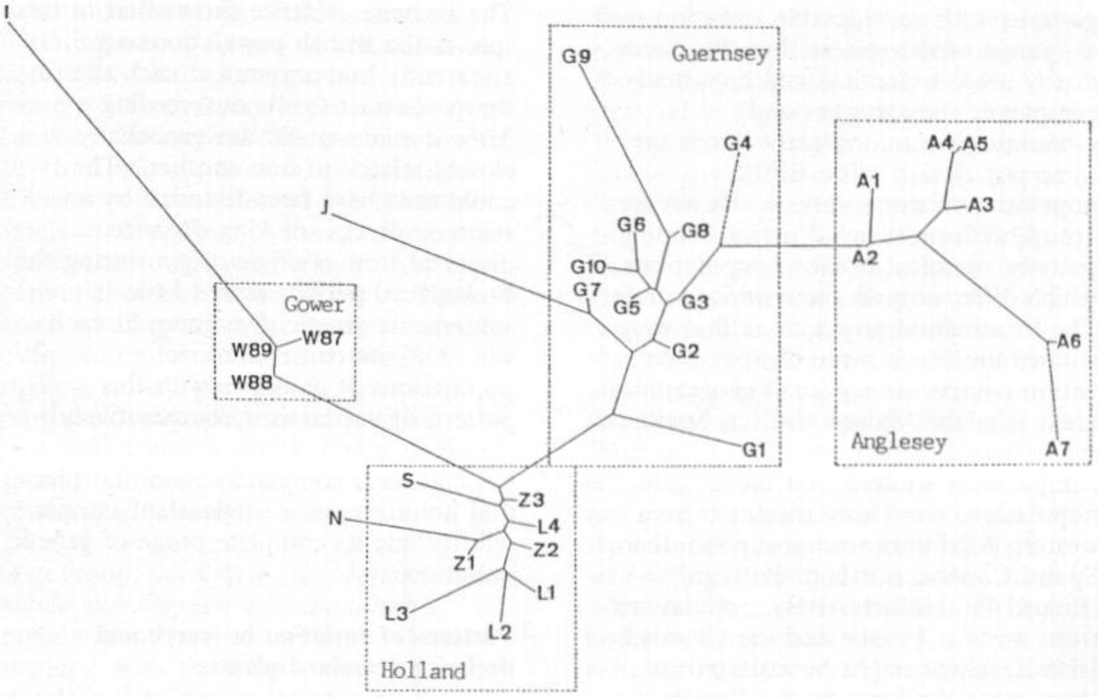

Figure 3 Unrooted phylogenetic tree showing relationships among populations and subpopulations of *Mibora minima* in Britain (W, Whiteford; A, Anglesey), the Channel Islands (G, Guernsey; J, Jersey) and Holland (L, Laren; S, Simpleveld; Z, Zundert). I is a commercially available stock, and N a naturalised population in a nursery in East Anglia (see text). Branch lengths are proportional to genetic distances.

Guidelines for conservation of *M. minima* in Britain are that each main wild population should be maintained in sufficient numbers to preserve its level of genetic variation and heterozygosity, and that the present pattern of variation should not be altered by interpopulation transfers; particular care should be taken with the small, isolated and very distinctive Whiteford population.

Small restharrow *Ononis reclinata* is a small annual legume with erect stems and pink flowers of moderate (5–10 mm) size which are relatively conspicuous when they are open, although it is self-fertile and normally self-pollinating. The leaves and stems have a distinctive indumentum of shining glandular hairs, and the plant is thus easily identifiable even at the seedling stage. Its reproductive capacity is relatively low (only *c.* 50–120 seeds in plants of average size) and the seeds have no apparent means of dispersal except pod dehiscence, which may flick seeds several centimetres from the parent plant. It is rare in Britain, where it grows in a few small and widely separated coastal sites. It is a chiefly Mediterranean species which extends along Atlantic coasts to Britain. Within its main Mediterranean range it is often patchily and discontinuously distributed, but is locally common in suitable areas.

Studies of chromosome number and isozyme variation were made on populations of *O. reclinata* from four apparently native British localities, in Devon, Glamorgan, Pembrokeshire and Wigtownshire, and from 11 Continental European sites (John 1992). A chromosome number of n = 16 or 2n = 32 was found in the three British localities for which counts were made, and at four Continental sites, with n = 15 or 2n = 30 at three more Continental sites and also in the closely related and possibly conspecific *O. dentata*. Nine enzyme systems were assayed. All showed multiple banding patterns with considerable variation over the range of the species, but they were nearly always identical and apparently completely homozygous and monomorphic among plants from the same population. The British populations form a very closely similar group, with most or all major banding patterns identical between populations, which differ only in some minor bands. The Continental populations that were studied are much more diverse, even within comparatively small geographical areas (e.g. the Málaga district, Spain, or the eastern Peloponnese, Greece); overall comparisons showed that the British populations were least distant from a western Mediterranean group (southern Spain, Corsica, northern Portugal) (Figure 4). Unfortunately, populations from western France and the Channel Islands, which might be expected to show most similarity to the British populations, could not be included in the study.

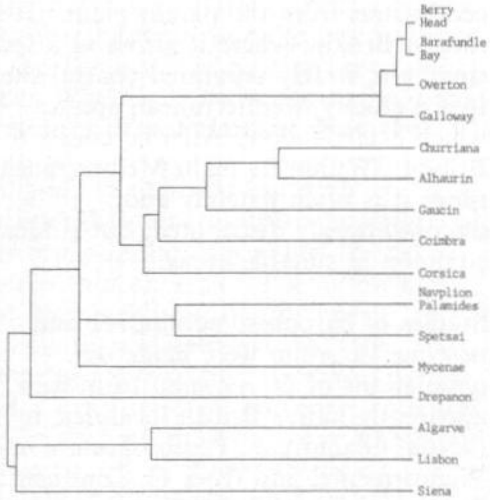

Figure 4 Dendrogram showing relationships among 15 British and European populations of *Ononis reclinata* and one (Lisbon) identified as *O. dentata*, based on pairwise comparisons of isozyme banding patterns. The scale units are % similarity.

The isozyme evidence shows that in this species the British populations are apparently homozygous at each site (in sharp contrast to the outbreeding *Mibora minima*) and are genetically closely related to one another. They could thus have been founded by a stepwise process of long-distance dispersal from a single origin during the Post-glacial period, with a little subsequent genetic divergence at each site. Off-site conservation of populations of a species with this pattern of variation is comparatively simple in genetic terms, because if a population is completely monomorphic and homozygous a single-plant sample will include its complete range of genetic variation.

Patterns of variation in scarce and declining grassland plants

Ancient lowland grasslands in Britain have been reduced to a patchwork of remnants by agricultural change, mainly during the last 50–100 years. Although they were largely dependent on traditional agriculture, they probably have many similarities to the natural grassland communities, intensively grazed by wild herbivores, of pre-agricultural times. Some of the most ecologically restricted and now increasingly scarce plants of these ancient grasslands are western European endemic species, adapted to the oceanic climate of Atlantic Europe. Others, for example *Salvia pratensis* and *Scabiosa columbaria*, have a more continental distribution in Europe and are at the western limits of their geographical and ecological ranges in British grasslands. Recent studies of *Salvia pratensis* and *Scabiosa columbaria* in Holland have suggested that genetic erosion of small isolated populations may be a significant factor contributing to the future decline of such grassland species there (Oubourg, van Treuren & van Damme 1991; van Treuren *et al.* 1991, 1993).

We have studied reproductive strategies, population structure and isozyme variation in a range of threatened or declining native species of grasslands and related habitats, especially the western European endemics whorled caraway *Carum verticillatum*, meadow thistle *Cirsium dissectum*, chamomile *Chamaemelum nobile* and wood bitter-vetch *Vicia orobus*. Although these four species are long-lived herbaceous perennials which appear to show similar levels of adaptation for predominant outbreeding, we found that there are striking differences in their functional reproductive strategies and in their patterns of variation (Table 1); the four species fall into two distinct and contrasting groups.

One group, consisting of *Chamaemelum nobile* and *Cirsium dissectum*, has a predominantly vegetative reproductive strategy, with the ability to establish stands and to persist largely or entirely by vegetative spread. Although both species can show high seed fertility under favourable conditions, seed production and recruitment of seedlings are commonly sparse or absent. Nevertheless, they show greater potential than the second group for long-distance dispersal of their seeds (*Cirsium dissectum* by wind, and the small seeds of *Chamaemelum nobile*, less certainly but over greater distances, by waterfowl visiting or grazing pondside swards). The two species in the other group, *Carum verticillatum* and *Vicia orobus*, have little or no ability to spread vegetatively, but flower regularly and often prolifically, with medium to high allocation to seed production and recruitment of seedlings. They show little or no potential for long-distance dispersal of their seeds under modern conditions, although in the past they may have been dispersed by far-ranging grazing animals.

Patterns of isozymically detectable genetic variation are sharply different in the two groups. *Chamaemelum nobile* and *Cirsium dissectum* both show little or no genetic variation in most of the populations that we studied. In *Chamaemelum nobile*, most Welsh populations appear to consist of only one isozyme genotype, sometimes identical in a pair of populations (Pilton Green and Reynoldston, West Glamorgan; Kennexstone and Burry Green, West Glamorgan) but usually differing between populations (Table 1). These monomorphic populations also show low or zero seed-set in the field, suggesting that each population may consist largely or entirely of a single self-incompatible clone, as in *Aster furcatus* (Les, Reinartz & Esselman 1991). One population (St. David's Head, Dyfed) was found to consist of two isozyme genotypes (perhaps two clones), and also showed fairly high seed-set on some heads; another (the extensive Porth-clais population, Dyfed) had a more normal pattern of variation, with several genotypes, and good seed-set on many heads. Interpretation of this pattern of variation is complicated by the possibility that some populations may have originated as escapes from cultivation, but the genotypic differences that exist between monomorphic local populations are also consistent with the expected pattern of variation in a species in which marginal populations persist vegetatively after being founded by single pioneers from genetically variable, seed-setting populations elsewhere (usually, but not always, within or nearer to the main range of the species). Subsequent work showed that genetically uniform populations of *C. nobile* likely to consist of one or a few clones also occur frequently in southern England, in addition to more variable populations of the Porth-clais type, with overall levels of variation increasing towards the south-east (Figure 5). However, self-fertility was found in at least some populations, suggesting that predominant vegetative reproduction rather than the absence of the ability to reproduce by seed may be at least partly responsible for the monomorphy that we found in many populations (Kay & John 1994, 1995).

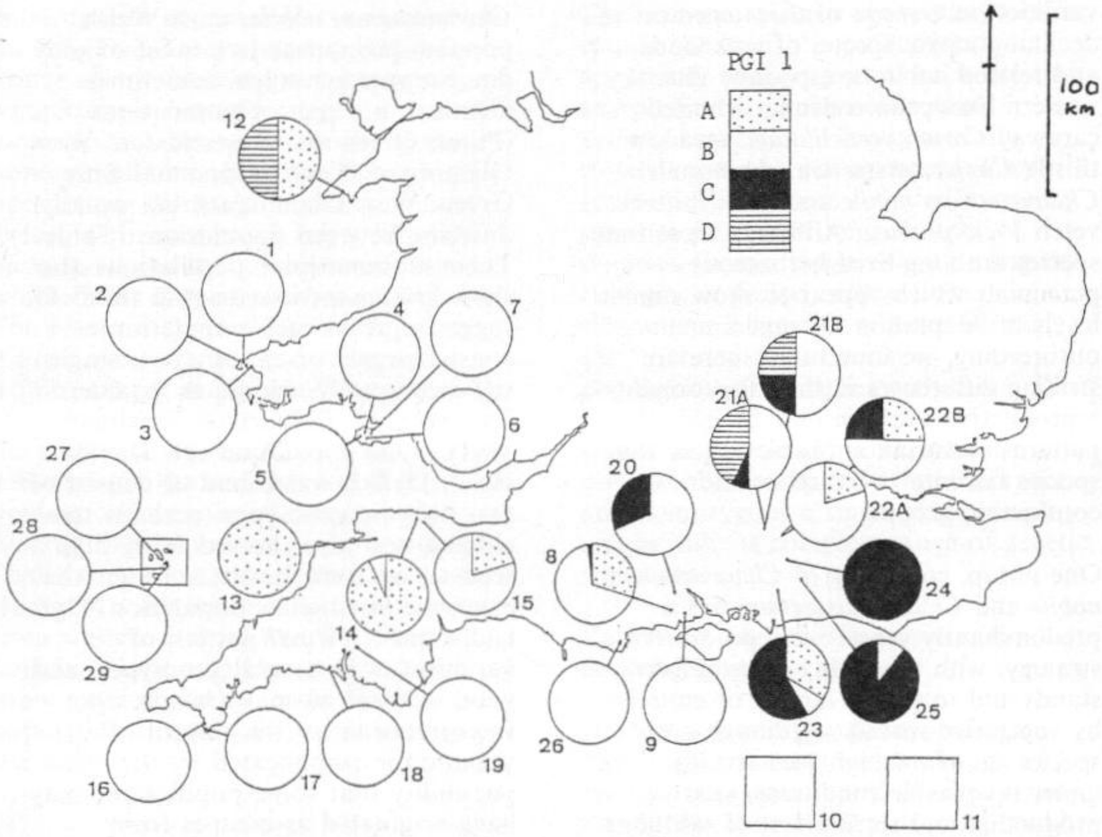

Figure 5 Allele frequencies in *Chamaemelum nobile* populations in Britain; phosphoglucose isomerase 1 *(PGI –1)*. The unlocated square symbols (10 and 11) represent the cultivars 'Flore Pleno' and 'Treneague'.

Cirsium dissectum is electrophoretically less easy to resolve than the other species among those under study, and at first we found it hard to accept that all ten populations that we initially analysed from its Welsh range, with up to 56 samples from a single population (Kenfig, Mid Glamorgan), were monomorphic and identical both within and between populations (Table 1). Improved techniques confirmed that this was indeed so at the eight loci that were clearly resolved, and the eventual discovery during 1992 of a population in southern England (Marlpit Oak in Hampshire) that was polymorphic, although at only one locus, suggested that this species might show extreme edge-of-range effects, with a single isozyme genotype occurring over a wide geographical area at the edge of its range in Wales and perhaps also in western England. *Cirsium dissectum* appears to reach its western ecological limit in Wales; marginal populations in Pembrokeshire and Cardiganshire are few and small, often consisting of a single apparently clonal patch. Despite this apparent limit, the species re-appears in Ireland, where it is widespread and abundant. Our tentative predictions that Irish populations might show isozymic variation were confirmed by sampling during 1993. We have found only two Welsh populations in which any detectable isozyme variability occurs, in one case probably as a result of a single-locus mutation and in the other possibly as a consequence of the morphologically similar F1 hybrid with *C. palustre* being included in the sample. In contrast, several Irish populations show significant although still moderate levels of isozyme variation (Table 1). Seed-set is, however, often quite high on the capitula that are produced (normally rather sparsely) in populations of *C. dissectum* in Wales, despite its general isozyme monomorphy there. Although it is clearly possible that a lack of genetic variation is at least partly responsible for the limitation of the range of this species in western Britain, alternative interpretations cannot be excluded (Kay & John 1994, 1995).

The implications of these reproductive strategies and patterns of variation for the conservation of *Chamaemelum nobile* and *Cirsium dissectum* are mixed. On the one hand, the apparent monomorphy and clonal nature of many (*Chamaemelum nobile*) or most (*Cirsium dissectum*) populations suggests that single-plant samples could represent their entire range of genetic variation; if this is correct, sampling, off-site maintenance, and re-establishment of similar populations during a 'recovery' programme would be comparatively simple. However, despite the lack of detectable isozymic variation, some variation may exist, which would be lost by these procedures. On the other hand, there are several indications that the ability of marginal populations of these species to persist or adapt may be limited by their lack of genetic variation; a case could be made out for increasing the range of genetic variation within a population, or in a newly established experimental population, by introducing or combining different genotypes, especially if these already exist in physically separated populations within the region.

The second group of species (*Carum verticillatum* and *Vicia orobus*) proved to have a totally different pattern of variation. In contrast to the genetic impoverishment or monomorphy of the local populations of the first group, they showed high levels of both genetic variation and heterozygosity in most samples, usually with little evidence of genetic erosion in the sense used by van Treuren *et al.* (1991, 1993) even in very small populations, although some populations of *Vicia orobus* did show genetic impoverishment and others showed wide deviations from expected levels of heterozygosity. It is likely that the longevity of individual plants is an important factor slowing down the process of genetic erosion in small remnant populations of this and other species.

Samples were tested from 41 populations of *Carum verticillatum* (Table 1), of which six were from Scotland, three from England and the remainder from Wales, and it was possible to make fairly full genetic comparisons between three of the main disjunct areas of distribution of this species within Britain (southern and central Wales, northern Wales, and south-western Scotland) and also between these areas and two outlying sites (Martin Mere, Lancashire, and Winkfield, Berkshire) and with one representative of the Devon/Cornwall disjunct area (Hollow Moor/Odham Moor). In addition, detailed genetic comparisons were possible within the first three areas. *Carum verticillatum* has a strong tendency to occur in more or less contiguous populations where suitable sites occur, both locally and in wider geographical areas, suggesting that some factor or factors may prevent the establishment or maintenance of isolated populations. Genetic distances between populations showed clear local groupings and a broad overall correlation with the main disjunct areas of distribution, but in all areas where several populations were investigated some were anomalously widely separated in terms of genetic distance, often being associated with other geographical groupings. There was some correlation between the genetic distances between populations and their geographical distance of separation. The populations from southern and central Wales were notably bimodal, one subgroup being close to or overlapping with the group from northern Wales, but this bimodality did not correspond to any obvious geographical groupings within southern and central Wales. The isolated Berkshire population was close to one of the subgroups within southern and central Wales, suggesting a possible correlation with hay or stock originating from this region, but the frequent exceptions to the correlations between genetic and geographical distance that we found make such connections very speculative.

There is some evidence of minor genetic erosion in *Carum verticillatum*, but at much lower levels than in the *Salvia pratensis* and *Scabiosa columbaria* populations studied in The Netherlands by van Treuren *et al.* (1991, 1993), probably as a result of larger population sizes and more effective gene-flow in *C. verticillatum* (Kay & John 1994). The reasons for the unusual pattern of distribution of *C. verticillatum* (fairly common within quite sharply defined geographical areas, absent or rare beyond these areas) are still unclear, but seem likely to result from ecological rather than genetic factors. Similar patterns of distribution occur in other members of the same family (Apiaceae), including species of *Oenanthe* (Perring & Walters 1976).

Indications for conservation of *C. verticillatum* are that population sizes should be kept fairly large in order to maintain existing levels of genetic variation, and that transfers should not be made between different areas.

Vicia orobus is pollinated by specialist long-tongued bumblebees, chiefly *Bombus pascuorum*, which are likely to bring about full pollen transfer and free gene-flow within populations (Kay & John 1994). Genetic studies of nine variable loci in seven polymorphic enzyme systems, in 21 populations of varying size, all in Wales, have shown that overall levels of genetic variation are high (Table 1, Figure 6). Gene diversity, total allele number and heterozygosity differed markedly among populations. Some comparatively large populations, notably a well-sampled (n = 35) population of several hundred plants at Llwyn Iarth in north Wales (population 14 in Figure 6), showed quite severe genetic impoverishment. At Llwyn Iarth, three of the seven normally polymorphic loci were monomorphic, and total allele number was substantially lower than in most other full samples. This may have resulted from founder effect or a past bottleneck; it highlights the importance of genetic screening of populations that might be used as sources of material for reintroduction, because, if population size alone had been considered, this large population might have appeared to be a particularly suitable source. The genetic data suggest that it would in fact have been a particularly bad choice.

Several small populations of *V. orobus* showed genetic structures that are likely to result from selective and stochastic effects related to rapid decline, restriction in numbers and neighbourhood size, and in some cases strong selection. There was some evidence of genetic erosion, but, despite the small numbers of plants that now exist in many declining populations, surprisingly high levels of genetic variation were often found to be retained, perhaps because the plants forming these populations are long-lived survivors rather than recruits from a depleted, inbred gene-pool, with strong selection adding to the retention of variation by favouring heterozygosity (Kay & John 1994).

Indications for conservation in this increasingly threatened species are that population sizes should be maintained at the maximum that is possible, and that careful genetic screening and monitoring are essential during any programmes that involve multiplication and re-establishment. In some cases at least, it may be desirable to intercross remnant local populations within a local area to re-establish the original levels of variation.

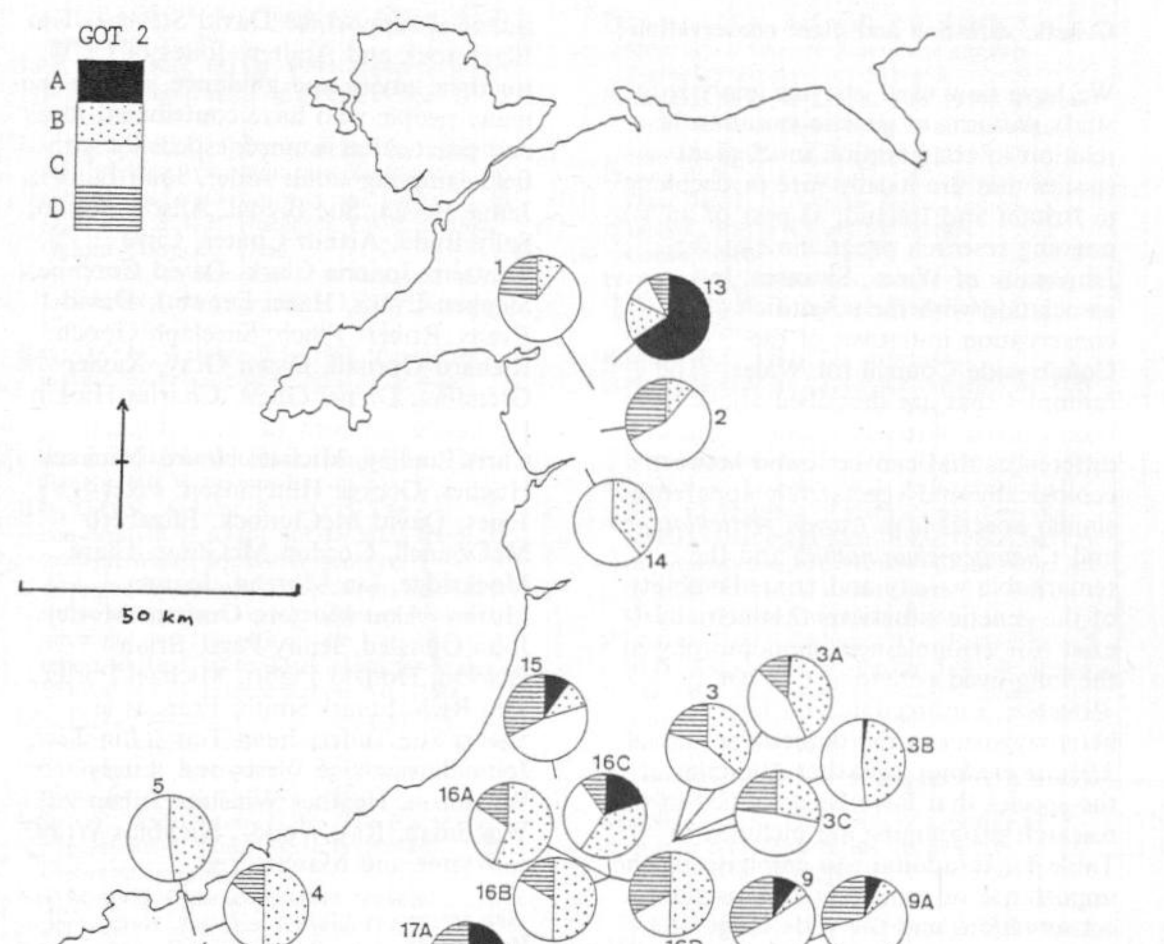

Figure 6 Allele frequencies in *Vicia orobus* populations in Wales; glutamate-oxaloacetate transaminase *2* *(GOT–2)*.

Genetic variation and plant conservation

We have now used isozyme analysis to study patterns of genetic variation in relation to conservation in 22 plant species that are locally rare or declining in Britain and Ireland, as part of an ongoing research programme at the University of Wales, Swansea, in association with the scientific conservation initiatives of the Countryside Council for Wales. The examples that are described above have been chosen partly to illustrate the wide differences that can be found between ecologically and vegetatively apparently similar species (e.g. *Carum verticillatum* and *Chamaemelum nobile*) and the remarkable variety and unpredictability of the genetic situations that actually exist (for example near-monomorphy in the long-lived perennial *Cirsium dissectum*, outbreeding and high heterozygosity in the diminutive annual *Mibora minima*). Further examples of the species that have been studied in this research programme are included in Table 1. In addition to emphasising the importance of genetic factors in plant conservation, and the wide range of breeding systems and consequent likely patterns of genetic variation that exist in plants (Falk & Holsinger 1991; Gray this volume) it is also necessary to emphasise the importance of studying each species and situation individually. Inferences and generalisations from apparently similar situations in other plant species, and even from other populations of the same species, are often inapplicable, sometimes dangerously so. Theoretical modelling can also be extremely misleading, even when its premises are correct. Knowledge of the reproductive strategy, breeding system and patterns of genetic variation in a plant species has a central role in any strategy for its conservation; this knowledge can be obtained only by direct study of that species.

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Genecology, the genetic system and the conservation genetics of uncommon British grasses

Alan Gray

Gray, A. 1997. Genecology, the genetic system and the conservation genetics of uncommon British grasses. *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, 56–64. Peterborough, JNCC.

Theory predicts that small population size has population genetic consequences resulting from inbreeding and drift. Yet population genetic structure is also determined by other processes resulting from the interaction between the plant's genotype and its environment (its genecology *sensu* Turesson 1923) and the various controls upon recombination, such as life-history, the mating system, and the chromosome system including meiotic behaviour (its genetic system, *sensu* Darlington 1958).

This paper draws on examples from the British grass flora to illustrate how these interacting processes can not only counter the effects of population size but also call for quite a different approach to the application of genetic principles to conservation. An approach based on sound ecological genetics principles is advocated, incorporating what is known about the species' genetic system with what may be expected to be the pattern of differentiation based on its spectrum of habitat-types, ecology and distribution – its genecology. From these it is possible to devise a realistic and achievable strategy for the conservation of genetic variation within declining or endangered species.

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Introduction

The title of this paper includes two expressions which, if not exactly old-fashioned, are rarely encountered in today's conservation genetics literature: *viz*. 'genecology' and 'genetic system'. Their definition here is important as a preface to the paper's central message – which is that anything less than a broad, holistic approach based on ecological principles can very readily lead to the view that genetics has only a subsidiary role in nature conservation.

'Genecology' was coined by the Swedish botanist Göte Turesson more than 70 years ago to refer to "the study of intraspecific variation (in plants) in relation to habitat" (Turesson 1923). The term is widely misunderstood – the 'gene' prefix is not from 'genetics' but from 'genos', the Greek word for 'race'. Turesson's 'race ecology' was not simply about the ecology and behaviour of genes in populations but about the whole complex of interacting processes which generate distribution patterns of heritable variation in plants. The term 'genetic system', *sensu* Darlington (1958), has a similarly broad ambit, and includes all those processes and factors which control, or constrain, the transmission of genetic information from one generation to the next. Such factors may be intrinsic, as are the chromosome, meiotic or breeding systems, or external, such as the spatial arrangement, or genetically-effective size, of populations.

That population size is only one of many factors affecting the genetic structure of species' populations immediately emphasises that an approach to the conservation of small, or endangered, populations based solely on 'beanbag' genetics is unlikely to be helpful. In this contribution I draw on examples from the British grass (Poaceae) flora to suggest that a broader approach, at the level of the species' genecology and incorporating as many of the interacting elements of its genetic system as possible, offers a more realistic framework for incorporating genetic principles and ideas into conservation policy and management.

Genetic structure and population size

The predicted population genetic consequences of small population size are well known. These arise chiefly from heightened levels of inbreeding and from genetic drift, but also include less obvious forces such as the influence of a reduction in compatible mating types. They involve loss of heterozygosity, loss of genetic diversity because of the (random) loss and fixation of alleles under drift, and the potential for greater between-population genetic differentiation. Despite this, broad generalisations about species which are found habitually in either large or small populations, or about the relative genetic diversity of large and small populations of the same species, cannot easily be drawn. This is not surprising for a number of reasons.

First among these is the mismatch between population size, N, and genetically-effective population size, N_e. The enormous variation in N_e/N ratios demands extreme caution in attempting to infer N_e from N. Ratios ranging from 0.01 to 0.95 (i.e. genetically effective population sizes between 1% and 95% of the census number) were listed by Crawford (1984) from the relatively few empirical studies (mostly on animals) then available. Grasses, and other clonal herbs, may present particular difficulties in this respect because extensive clonal spread in many species makes it difficult even to count individuals (i.e. genetically separate individuals or 'genets'). In many cases, extensive grass swards may consist of only one or a few clones and, in the extreme example of *Spartina anglica*, a single individual may cover entire stretches of coastline (Gray, Marshall & Raybould 1991; Raybould *et al.* 1991). The numbers of genets in a sward are also likely to vary between populations, as was demonstrated by Gray, Parsell & Scott (1979) in the saltmarsh grass *Puccinellia maritima*. Using isoenzyme variation to identify clone structure, an ungrazed marsh was found to have more than twice as many individuals per unit area of sward than a closely-adjacent marsh grazed by sheep.

Even where it is possible to count individual plants, the many simplifying assumptions of theoretical population genetics are so rarely met by natural plant populations that their effective size is frequently difficult to estimate. Variance in plant density, pollen- and seed-dispersal distances, sex ratios, outcrossing rates, fluctuations in population sizes, family sizes (progeny numbers) and that arising from overlapping generations all affect genetically-effective size. None of these parameters is impossible to estimate in a detailed study and there are appropriate formulations for each to relate N to N_e. Nonetheless, collectively they prevent N_e being superficially derived from N.

A second reason (or group of reasons) why population size and genetic structure are rarely straightforwardly related is that size is but one of the many factors impacting the processes of inbreeding and drift. Other aspects of the plant's ecology, demographic structure and the genetic system are at least (and often more) important. A simple illustration is given in Figure 1, which shows variation in rates of loss of heterozygosity under (a) various mating systems, and (b) a range of ploidy levels. The effects of these two aspects on the potential decay of heterozygosity in a founder population are profound (e.g. by comparing (a) and (b) it can be seen

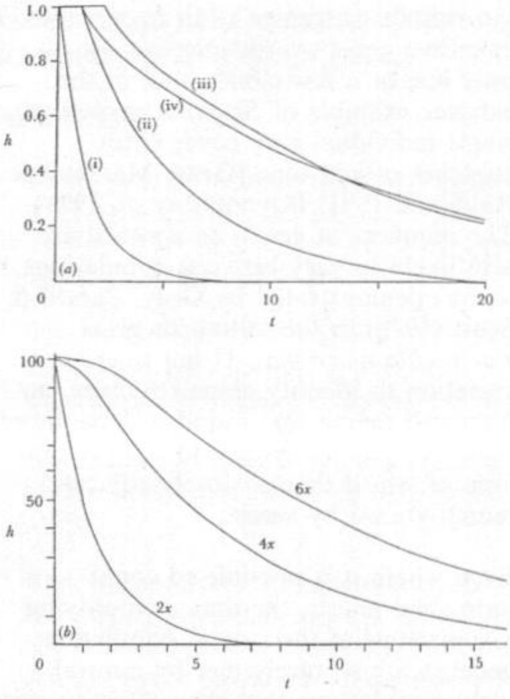


Figure 1. Loss of heterozygosity under various mating systems and ploidy levels. (a) Loss of heterozygosity on inbreeding in diploids with (i) self-fertilisation, (ii) sib-mating, (iii) double first-cousin mating, and (iv) circular half-sib mating. (b) Loss of heterozygosity on selfing in diploids (*2x*), autotetraploids (*4x*) and autohexaploids (*6x*). (The *y*-axis is the heterozygosity relative to the starting population. The *x*-axis is the time in generations.) (After Gray 1986.)

that selfing in autotetraploids is roughly equivalent to sib-mating in diploids). Further aspects of the genetic system, such as meiotic behaviour, which differentially affect recombination, and hence population genetic structure, are mentioned below.

These factors combine to make it difficult to predict the impact of small population size on population genetic structure and viability. A sudden reduction in size, as in a colonising (founder) population or by accident, isolation or rapid demographic decline (a population bottleneck), will have very different consequences in different species' populations, and the very limited number of experimental investigations of inbreeding in natural populations, such as the work of Lacy and his colleagues on *Peromyscus* deer mice (Lacy 1992), have underlined our lack of knowledge of the genetic basis of inbreeding effects (see also Gray (1996) and papers in this volume by Ouborg & Van Treuren and Tremayne & Richards). Although populations that habitually inbreed may be expected to be less vulnerable to inbreeding depression than regular outbreeders, differences in historical inbreeding rates are not reliable predictors of the outcome of further rounds of inbreeding. Furthermore, some typically selfing species of plant suffer from strong inbreeding depression (Barrett & Kohn 1991).

Conservation genetics

Despite the difficulties outlined above, Ellstrand & Elam (1993) have attempted to apply population genetic principles to plant conservation by identifying those circumstances in which plant species and populations are at 'genetic risk'. Reviewing the evidence, they point to warning signs of population vulnerability such as sudden changes in population size, or degree of isolation or fitness. Using this approach, they suggest a number of management strategies appropriate to the genetic 'problem', e.g. reducing gene flow where this is having, or may lead to, a deleterious effect – say in cases of hybridisation of rare plants with common congenerics, or where there is evidence of outbreeding depression.

Below, I present an alternative approach, which may be helpful in situations where even minimal genetic information is unavailable. This involves an analysis of all that is known about the species' ecology, genecology and genetic system, and an attempt to place this in the context of the broader evolutionary patterns and trends within the genus or family.

An introduction to the example group of species, uncommon British grasses, is provided by the maps in Figure 2. These illustrate the strongly contrasting

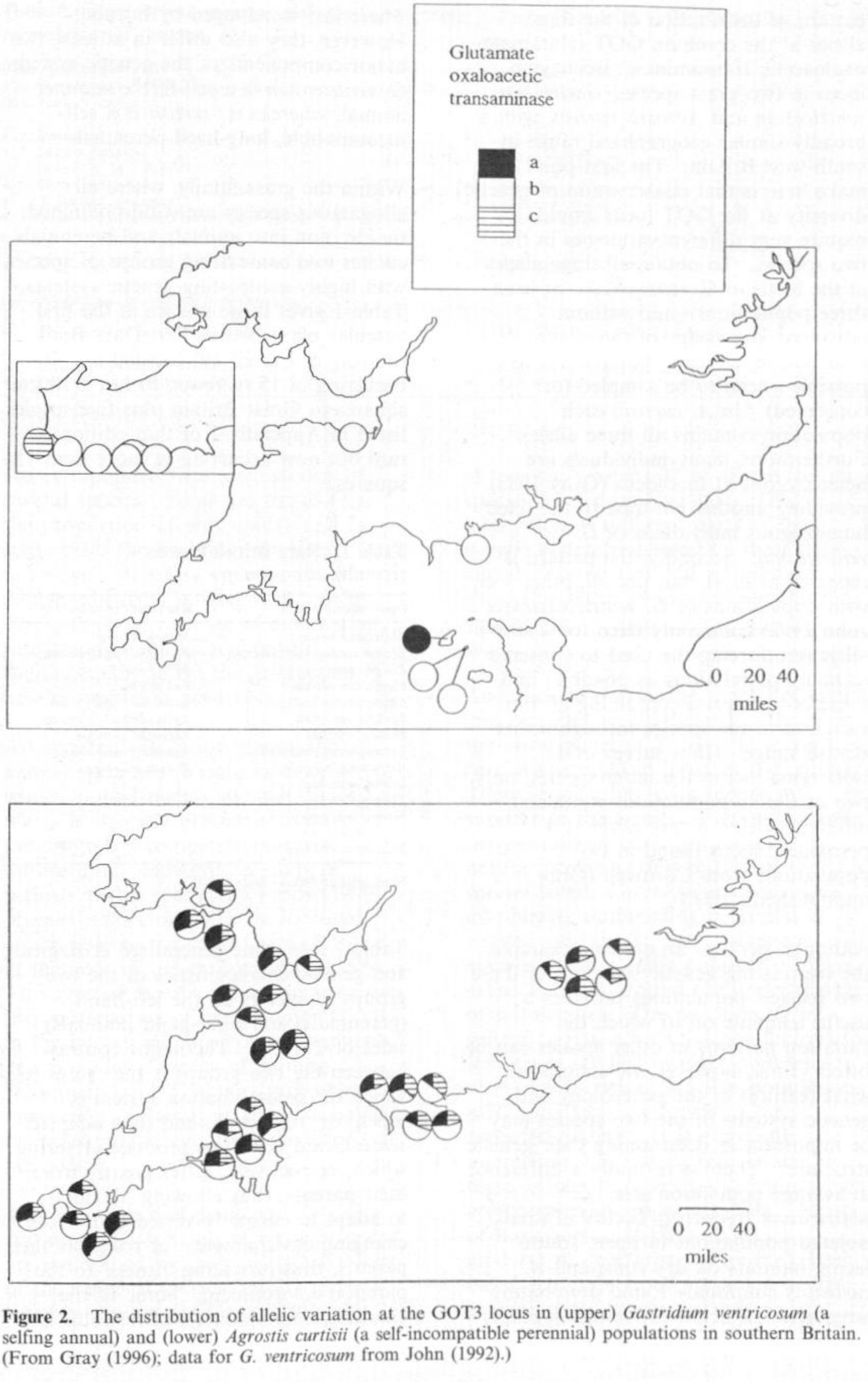


Figure 2. The distribution of allelic variation at the GOT3 locus in (upper) *Gastridium ventricosum* (a selfing annual) and (lower) *Agrostis curtisii* (a self-incompatible perennial) populations in southern Britain. (From Gray (1996); data for *G. ventricosum* from John (1992).)

pattern of distribution of the three alleles at the common GOT (glutamate oxaloacetic transaminase) isoenzyme locus in two grass species, *Gastridium ventricosum* and *Agrostis curtisii*, with a broadly similar geographical range in south-west Britain. The first point to make here is that conservation of genetic diversity at the GOT locus would require very different strategies in the two species. To obtain all three alleles at the locus in *G. ventricosum*, at least three populations – and without advanced knowledge of the allelic distribution, as many populations as possible – need to be sampled (or conserved). In *A. curtisii*, each population contains all three alleles. Furthermore, many individuals are heterozygous at this locus (Gray 1988), providing another contrast to the fixed homozygous individuals of *G. ventricosum*. Secondly, the pattern is repeated at most, but not all, other loci, with populations of *G. ventricosum* (see John 1992) commonly fixed for a single allele (reinforcing the need to conserve as many populations as possible) and those of *A. curtisii* containing all the variation in the species throughout its British range. (In a survey of 11 isoenzyme loci in the latter species, only two of the 30 populations contained different alleles, a rare allele at a peroxidase locus found in two populations from Cornwall (Gray unpublished data).)

Although perhaps an extreme example, the contrasting genetic structure of these two species' populations provides a useful template on to which the variation patterns in other species can be fitted. First, however, we should ask what features of the genecology and genetic systems of the two species may be important in determining their genetic structure? There is certainly a difference in average population size. *G. ventricosum* frequently occurs in small, isolated populations in open, south-facing habitats on dry soils, and *A. curtisii* is commonly found dominating large areas of acid heathland, especially where this is managed by burning. However, they also differ in at least two major components of the genetic system: *G. ventricosum* is a self-fertile summer annual, whereas *A. curtisii* is a self-incompatible, long-lived perennial.

Within the grass family, where all allogamous species are wind-pollinated, the division into annuals and perennials creates two contrasting groups of species with highly contrasting genetic systems. Table 1 gives those species in the first vascular plant British Red Data Book (Perring & Farrell 1977) listed as occurring in 15 or fewer 10 km x 10 km squares in Great Britain plus five species listed in Appendix 1 of that edition as rare but now occurring in more than 15 squares.

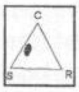
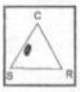
Table 1. Rare British grasses – recombination groups

Open recombination	Restricted recombination
Alopecurus borealis	*Mibora minima*
Elymus caninus var. *donianus*	*Polypogon monspeliensis*
Poa flexuosa	*Briza minor*
Hierochloe odorata	*Poa infirma*
Calamagrostis scotica	*Gastridium ventricosum*
Koeleria vallesiana	*Anthoxanthum aristatum*
Festuca arenaria	*Anisantha tectorum*
Corynephorus canescens	*Anisantha madritensis*
Alopecurus bulbosus	*Bromus interruptus*
Leersia oryzoides	
Phleum phleoides	
Festuca longifolia	
Cynodon dactylon	
Spartina alterniflora	

Nomenclature follows Stace (1991)

Table 2 lists some generalised ecological and genetic characteristics of the two groups of species on the left-hand (perennials) and right-hand (annuals) sides of Table 1. The major contrast between the two groups is the extent to which the recombination system is 'open' or 'restricted', and thus adapted *sensu* Grant (1958) to produce offspring which, respectively, differ greatly from their parents, thus allowing 'flexibility' to adapt to currently varied and future changing environments, or resemble their parents, thus providing 'fitness' to the parental environment. Some of the characteristics summarised in Table 2

Table 2. Rare British grasses: ecological and genetic features of species with contrasting recombination systems

Open recombination	Restricted recombination
(a) Features known:	
• Perennials	• Annuals
• Largely polyploid (x = 40.2) 14% diploids	• Largely diploid (x = 17.7) 67% diploids
• Outbreeding	• Inbreeding (selfing)
• 'K-selected' strategists	• 'r-selected' strategists
• Stress-tolerators/competitors (Grime 1979): 	• Ruderals (Grime 1979): 
• Mainly closed swards	• Open habitats
(b) Features mostly predicted from the above:	
• Many heterozygous loci individual^{-1}	• Many homozygous loci individual^{-1}
• Variable offspring (low recruitment)	• Uniform offspring (high recruitment)
• Between-population variance low	• Between-population variance high
• Unit of variation = the genotype	• Unit of variation = the population

x = mean chromosome number

are extrapolated from case studies of several species. Some are trends (e.g. the proportion of polyploids), and in some cases the breeding system is unknown. Breeding systems are, of course, not fixed attributes of species. The occasional increase in outcrossing rate among habitual selfers will fundamentally affect the production of new recombinant genotypes.

An apparent exception among the annual species is *Mibora minima*, a winter annual mainly of sand dunes which is strongly protogynous and (although self-compatible) largely outbreeding. However, a study of meiosis in this species by Ferris (1990) revealed that chiasmata are localised either close to the centromere (75%) or at the ends of the chromosomes (25%). This type of meiotic behaviour promotes the conservation of genic combinations in the interstitial chromosome segments, and provides a means of limiting recombination. This may protect an outbreeder occurring in unstable habitats or small populations from the effects of inbreeding.

The importance of genic and chromosomal controls of recombination are underlined by the pairing behaviour of the range of species, mostly grasses, listed by Callow (in Gray 1986). All 23 inbreeding species formed only bivalents at meiosis, whereas 37 of 48 outbreeding species formed multivalents. The interaction of different parts of the genetic system suggested by these data, and the example of *Mibora minima*, should be borne in mind in ascribing species to particular recombination types.

As indicated above, the genetic structure and recruitment characteristics listed in the lower panel of Table 2 have yet to be demonstrated in most British grasses. There is some limited evidence in support of the trends. Figure 3 shows the patterns of variation revealed by natural progeny trials in two of the species listed, *Corynephorus canescens*, an obligate outbreeding perennial of coastal sand dunes, and *Polypogon monspeliensis*, a selfing annual of open, saline habitats around the upper fringes of saltmarshes. The partitioning of genetic variance between populations for four traits studied in eight populations of *P. monspeliensis* and five populations of *C. canescens* conforms to the expected pattern. It is especially interesting that this pattern is seen in these species in quantitative traits which are presumably under selection. Most of the evidence to date on which analyses of genetic structure have been based has been from presumed neutral marker genes (predominantly isoenzymes). As several others (e.g.

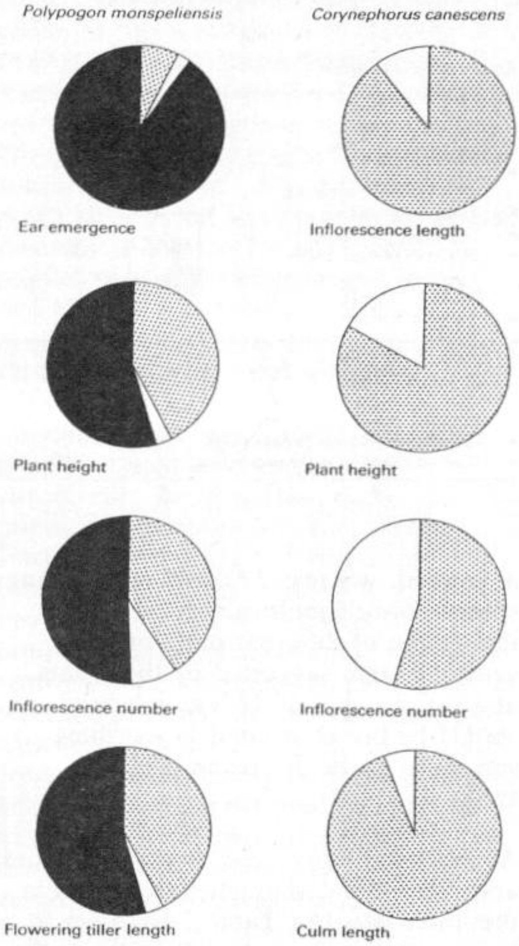


Figure 3. The partitioning of genetic variance between plants within families (speckled), between families (white), and between populations (black) in two grasses, *Polypogon monspeliensis* (left), a self-compatible annual, and *Corynephorus canescens*, a self-incompatible perennial. (After Gray 1991.)

Ennos *et al.* this volume) have pointed out, evidence of genetic diversity based on isoenzymes may be at variance with that based on quantitative traits under polygenic control.

Conservation of small and endangered populations

The burgeoning isozyme literature and the increasing application of molecular methods to detect variation at the DNA level continue to improve our picture of the distribution of genetic variation in natural populations. Many specific examples of species conservation or recovery, either *in situ* or by captive breeding, are being illuminated by a knowledge of genetic variation and the application of population genetic principles. Yet for the vast majority of species, even those which are rare, we have no information on the genetic structure of their populations. Nor are we likely to find the time or resources to carry out more than a few rudimentary allozyme surveys (cf. Kay & John this volume). Where such surveys are possible, they are likely to prove extremely valuable in choosing appropriate conservation strategies, but will in some cases (e.g. Ennos *et al.* this volume) give a misleading picture of the genetic variation within the species.

In these circumstances, it may be desirable to take a broad approach similar to that outlined above and illustrated by a group for which the life-history features, chomosome numbers and (in several instances) breeding systems are known in addition to some general aspects of the species' ecology. Such an holistic approach can provide a guide to managers in deciding on the appropriate conservation strategy. The decisions as to how many, and even which, populations to conserve or sample for gene banks, and whether to defend a particular population of a rare or declining species, are likely to depend on what proportion of the total species' diversity the population in question is likely to hold. It turns out that a remarkably small sample of plants is likely to conserve all, or nearly all, the polymorphic genes segregating in a population, providing that their frequency is not less than 0.05 (Lawrence, Marshall & Davies 1995).

Finally, however, it is necessary to return to the Turessonian concept of genecology. The net effect of the genotypical response of plant species to their habitats was observed to be the production of populations closely 'adapted' to their environment – the 'ecotypes'. The widespread observation of such genotype-environment correlated variation in numerous genecological studies in the past 70 years (e.g. Briggs & Walters 1984) suggests that, other things being equal, a guiding principle of conservation, and one which incorporates the soundest of evolutionary and genetic principles, should be to sample and conserve those populations which represent the species' full spectrum of habitats. This will include populations at the extremes of the species' geographical range and across a range of habitat-types.

For example, among the British grasses in Table 1, it would be sensible to ensure the protection of representatives of the coastal dune populations and of the inland populations of *Corynephorus canescens*; of the Loch Leven, lowland wet fen and coastal saltmarsh populations of Scottish *Hierochloe odorata*; of Anglesey, Gower and Channel Islands populations of *Mibora minima*; of limestone and chalk grassland populations and (the now extremely rare) arable margin populations of *Gastridium ventricosum*, and so on. In a few cases the imperative of possible extinction demands that all populations are conserved, or sampled for *ex-situ* protection – as in the last known population of the accidentally introduced *Spartina alterniflora* in Southampton Water (other populations of this North American species and its subspecies *S. alterniflora* ssp. *glabra* in Southampton Water, Essex and Scotland are deliberate introductions at known dates since the evolution at the end of the last century of the hybrid *S. anglica* of which *S. alterniflora* is a known parent). For yet other species, e.g. *Polypogon monspeliensis*, *Cynodon dactylon*, the widely scattered and ephemeral populations represent the edge of a very extensive world distribution, and are thus likely to be of interest genetically.

For most species, however, the idealistic notion that all populations of all uncommon, especially declining, species must be defended ought to be replaced by a conservation strategy based on the principle that the pattern of genetic differentiation is likely to reflect the genotypic response to the local environment. Although dependent on and constrained by the genetic system, this response may provide a different dimension of variation. Thus, an approach based on the combination of genecological considerations and a preliminary analysis of the genetic system has much to offer in devising a realistic and achievable strategy for the conservation of genetic variation within rare and endangered species.

Such an approach may not be universally applicable and will be scale-dependent. For example, most temperate zone trees have similar breeding systems (monoecious, wind-pollinated, allogamous and self-incompatible) and other factors are more likely to influence the genetic structuring of their populations. Nevertheless, the broad relationships drawn by Hamrick and others (e.g. Hamrick & Godt 1989) between the genetic diversity in plant species' populations (as measured by isoenzymes) and a range of ecological (especially life-history) and breeding system parameters provide encouraging support for the approach advocated here.

Acknowledgements

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Genetic variation and population size in the rare dioecious plant *Silene diclinis* (Caryophyllaceae)

Honor C. Prentice and Stefan Andersson

Prentice, H.C., & Andersson, S. 1997. Genetic variation and population size in the rare dioecious plant, *Silene diclinis* (Caryophyllaceae). *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, 65–72. Peterborough, JNCC.

Silene diclinis is endemic to an area approximately 15 km × 6 km near the town of Xàtiva in the Spanish province of Valencia. The species is unusual among narrow endemics in that it is dioecious. There are fewer than ten extant populations of *S. diclinis*, and the populations are fragmented, isolated and small, usually containing fewer than 500 individuals. Low effective population sizes and spatial isolation between populations and subpopulations suggest that the structure of genetic variation in *S. diclinis* will reflect the effects of genetic drift. Preliminary results from studies of allozyme variation show, as predicted, a patchy distribution of alleles between populations and subpopulations, and significant relationships between the total number of alleles and the effective size of the population or subpopulation. The hierarchic structure of allozyme diversity and quantitative variation in floral characters is similar. The proportion of the total genetic diversity that is explained by differences between populations and subpopulations is relatively low. Between-family differences account for a relatively large proportion of the total variation (means of 18% and 28% for allozyme loci and quantitative characters, respectively). Most of the variation (quantitative characters 62%, allozymes 71%) is accounted for by within-family variation. Despite the similarity between the hierarchic partitioning of diversity in allozyme and quantitative characters, the two types of data show somewhat different patterns of geographic variation. And, although the majority of the genetic diversity is stored within populations, different populations and subpopulations contain different combinations of rare alleles. A conservation strategy that does not give priority to the maintenance of the different subpopulations at each site will lead to the loss of genetic variation in *S. diclinis*.

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Introduction

Silene diclinis: distribution and ecology

Silene diclinis (Lag.) Laínz is a diploid, perennial herb and is unusual among narrow endemics in being dioecious, with separate male and female individuals. The species is only found in an area approximately 15 km × 6 km near the town of Xàtiva in the Spanish province of Valencia (Figures 1 & 2) where it is restricted to a complex of low mountain ranges within an expanse of intensively cultivated lowlands (Prentice 1976; Mansanet & Mateo 1980; Prentice 1984). The species occurs either in semi-natural habitats where unstable slopes create a mosaic of occasional disturbance and open patches within the matorral scrub, or on the margins and terraces of old, traditionally managed groves. The rapid changes in land use within the area during the last decades are successively reducing the availability of suitable habitats. The disappearance of extensive grazing and abandonment of small-scale grove cultivation have led to vigorous matorral expansion, while some sites have been lost as a result of intensified agriculture. Several populations of *S. diclinis* that were

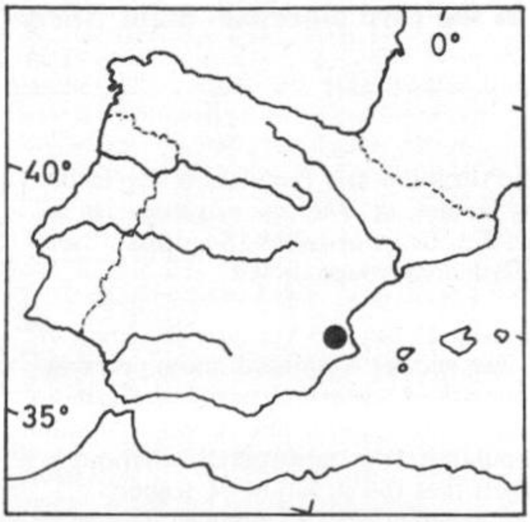

Figure 1. Map of the Iberian peninsula showing the endemic distribution of *Silene diclinis* in the Province of Valencia, Spain.

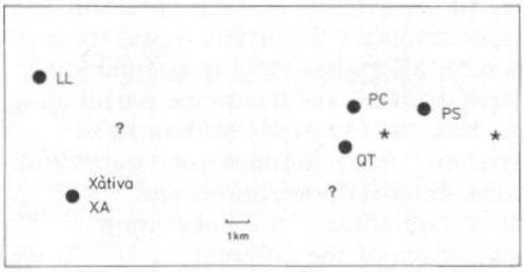

Figure 2. Distribution of *Silene diclinis* in the area around Xàtiva, Province of Valencia, SE Spain. • = sampled populations, ⋆ = populations not refound in 1995 and presumed extinct, ? = populations of uncertain status. The population codes are explained in Table 1.

known during the 1970s and 1980s appear to be extinct (J. Nebot, I. Mateu & H.C. Prentice pers. obs. 1995).

There are probably fewer than ten extant populations of *S. diclinis*, and the populations are fragmented, isolated and small. None of the populations is larger than 1000 individuals and several populations consist of fewer than 100 individuals. Most of the populations are subdivided into spatially disjunct subpopulations, separated by topographical or land-use barriers. Subpopulation sizes range from tens of individuals to a maximum of around 600 individuals (Table 1). There are probably fewer than 3000 individuals in total in the known populations of *S. diclinis* (J. Nebot, I. Mateu & H.C. Prentice pers. obs. 1995).

Population fragmentation, effective population size and genetic variation

Genetic drift is the random change in allele frequencies that occurs between generations as a result of sampling error in small populations. Genetic drift leads to the loss of rare alleles and a reduction in gene diversity within populations, as well as increased levels of divergence between populations (Nei, Maruyama & Chakraborty 1975; Brakefield 1989; Ellstrand & Elam 1993). Such non-selective, stochastic processes are expected to be important determinants of the structuring of genetic variation in *S. diclinis*, with its

Table 1. Information on populations of *Silene diclinis* used in allozyme and quantitative genetic studies. Counts of flowering individuals and estimates of total population sizes in the wild were made in the Xàtiva population in 1994 and in the rest of the populations in 1993. Sample sizes refer to the number of cultivated individuals used in the allozyme study (n_{enz}) and the number of cultivated families used in the quantitative genetic analysis (n_{fam}).

		Estimated total no. individuals	No. flowering individuals		Sample size	
Population	Subpopulation		males	females	n_{enz}	n_{fam}
La Llosa	LL	100	48	41	32	3
	LL-A	30	9	12	24	15
Xàtiva	XA-A	600	197	106	20	–
	XA-C	20	9	5	9	–
	XA-D	150	66	43	14	7
Pla de Suros	PS	300	145	79	42	2
	PS-A	30	13	6	9	5
	PS-B	150	72	43	19	10
Quatretonda	QT	300	84	126	81	5
Pla de Corrals	PC	50	12	20	28	5

fragmented and small populations. The fact that *S. diclinis* is dioecious means that the genetically effective number of breeding individuals within populations and subpopulations is even smaller than the census number of individuals. Effective population size will be reduced further by, for example, the presence of non-flowering individuals, differences in phenology and the emasculating effect of the anther smut *Ustilago violacea* (Pers.) Rouss. (cf. Lande & Barrowclough 1987).

An earlier study of within-population genetic structure in *S. diclinis*, at its *locus classicus* at Xàtiva, detected low levels of allozyme variation and a spatial structuring of allelic variation between disjunct subpopulations (Prentice 1984). The present study presents preliminary analyses of genetic data from a more extensive survey of variation – including material from the majority of the known populations and subpopulations of *S. diclinis*. We compare the structure of genetic variation in allozymes and quantitative genetic traits, and examine the extent to which patterns of geographic differentiation and the partitioning of genetic diversity can be interpreted in terms of random genetic drift within small populations. We also explore the relationship between effective population size and levels of allozyme variation within populations and subpopulations of *S. diclinis*.

Materials and methods

Field sampling and population censusing

Data on the sampled populations are presented in Table 1. Seed samples were collected from the five known populations of *S. diclinis* in 1991–1993. Populations LL, XA and PS (Figure 2) are subdivided into spatially separate subpopulations, isolated from each other by up to 1 km. These three populations are each represented by two or three, separate, subpopulation samples, whereas the continuously distributed populations, QT and PC, are each represented by a single sample (Table 1). Within subpopulations, seeds were collected from spatially separated female individuals. Dense, intermingled clumps of individuals were represented by a sample from a single female. Population censuses were carried out in 1993 and 1994 (by J. Nebot, Valencia). The total numbers of flowering male and female individuals were counted for each subpopulation. Estimates of the total numbers of flowering and vegetative individuals were also made. Effective population sizes were calculated for both subpopulations and populations, using the following equation:

$$N_e = \frac{4N_m N_f}{(N_m + N_f)}$$

where N_e is the effective population size and N_m and N_f are the census counts of males and females, respectively (see e.g. Hartl 1981). This parameter sets an upper limit for N_e and does not account for other factors, such as limited gene flow within subpopulations, fungal infection and phenological variation, which may also influence the number of breeding individuals (Lande & Barrowclough 1987).

Allozymes

Progeny families from the field-collected seeds were grown in an unheated greenhouse. A total of 278 individuals, belonging to 70 maternal families from ten subpopulations and five populations (Table 1), was scored for electrophoretic variation at eight polymorphic loci; *Dia–1*, *Pgi–2*, *Pgm–1*, *Pgm–2*, *Tpi–1*, *Est–1*, *Aat–1* and *Aat–2*, with a total of 22 alleles (H.C. Prentice, unpubl.). Allelic diversities were estimated using Nei's (1973) gene diversity statistic, *H*. The total mean (over the eight loci) allelic diversity was partitioned hierarchically (cf. Nei 1973; Chakraborty *et al.* 1982) into its within- and between-population, subpopulation and family components. Mean values (over loci) for *H* were calculated for each population and subpopulation.

The pattern of allele frequency differentiation between subpopulations was summarised using Ward's method of cluster analysis, based on Rogers' (1972) genetic distance.

Quantitative characters

Seeds from 52 families, representing five populations and eight subpopulations (Table 1), were sown on moist filter paper in Petri dishes. Twelve randomly chosen seedlings per maternal family were planted in pots with standard soil and placed in a randomised pattern in an unheated greenhouse. A large number of characters was measured on each plant, but here we focus on variation in a subset of seven floral traits; flowering date, flower colour (quantified with a home-made colour chart), pedicel length, petal length, petal width, petal claw length and calyx length. Flowering date and pedicel length were log-transformed to meet assumptions of parametric analyses. The sex of each flowering plant was also determined.

Nested analysis of variance (ANOVA) was performed for each trait to partition the total phenotypic variance into components attributable to population, subpopulation, family and within-family variation. These analyses were performed separately for males and females which differed significantly for all characters (S. Andersson & H.C. Prentice, unpubl.). The between-family component of variation indicates the extent to which different characters are genetically variable within populations.

The between-subpopulation pattern of differentiation in the flower characters was summarised using Ward's method of cluster analysis. The cluster analysis was based on Mahalanobis' distances derived from a canonical variates analysis (CVA) with the subpopulations as groups and individuals as replicates. Confounding effects of differences between males and females were removed by including sex as an additional group variable in the CVA.

Results

The hierarchical partitioning of diversity

The allozyme and quantitative partitions reflect a similar organisation of genetic diversity (Table 2). The between-population and -subpopulation components of diversity are relatively low in both types of character. The majority of the total diversity (62–71%) is explained by variation within families, and the between-family component of variation is relatively high (18–28%).

Table 2. Hierarchic partitioning of allozyme diversity (averaged over eight polymorphic loci) and variation in quantitative floral characters (averaged over sexes and characters) in *Silene diclinis.* Values are given as percentages of the total diversity.

Level	Allozymes	Quantitative characters
Between populations	6.5	4.6
Between subpopulations	4.2	5.1
Between families	18.2	28.2
Within families	71.1	62.1

Differentiation between subpopulations

The allozyme dendrogram (Figure 3) shows a tendency for the western and eastern (cf. Figure 2) populations to form geographic clusters. At a lower level in the dendrogram, within the major groupings, a subpopulation is more likely to cluster with subpopulations belonging to other populations than with other subpopulations belonging to its own population. There is thus a geographically patchy pattern of allele frequency differentiation between subpopulations. Quantitative genetic characters also reveal a patchy pattern of differentiation, with no geographic structure at the population or subpopulation level (Figure 3).

Relationships between effective population size and allozyme variation

Effective sizes (N_e) for subpopulations ranged between 13 and 276, whereas

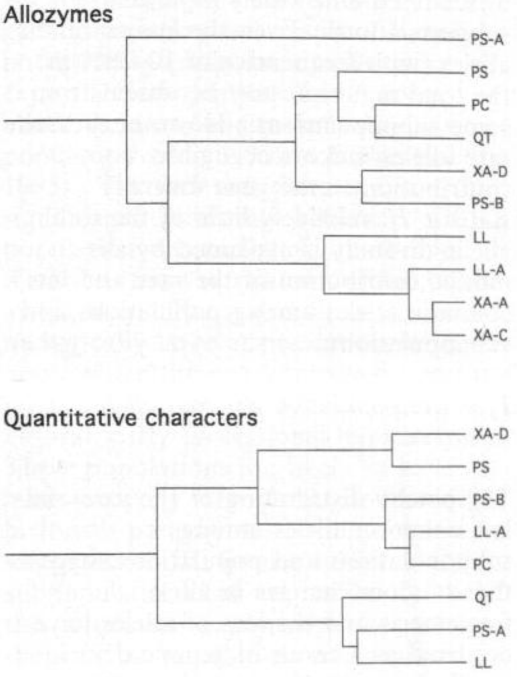

Figure 3. Geographic variation between subpopulations of *Silene diclinis*. The dendrograms were produced using Ward's method of cluster analysis. The 'Allozymes' dendrogram is based on Rogers' genetic distances (for eight polymorphic loci). The 'Quantitative characters' dendrogram is based on Mahalanobis' distances derived from a CVA using seven floral characters (after the removal of the effects of plant sex). The subpopulation codes are explained in Table 1.

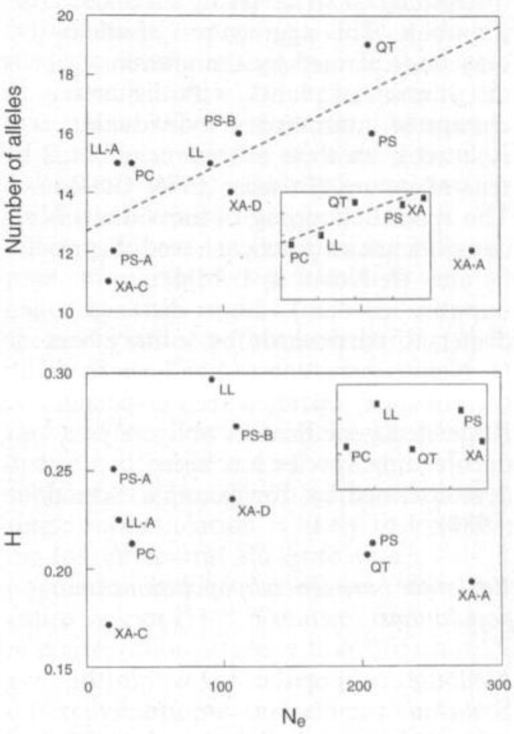

Figure 4. The relationship between effective population size (N_e) and allozyme variation within populations and subpopulations of *Silene diclinis*. The main figures show data from the ten subpopulations and the inserts show data from the five populations. The scale marks on the inserts are in the same units as those in the main figures. In the upper figure, "Number of alleles" is the allele count at eight polymorphic loci. In the lower figure, H is the mean allelic diversity (over eight polymorphic loci). Regression lines with slopes significantly ($P < 0.05$) greater than zero are shown by dashes.

population N_e ranged from 30 to 393 (Figure 4). The range of allele counts for subpopulations and populations respectively were 11–19 and 15–20 (Figure 4). Within-subpopulation allelic diversity (H, averaged over eight loci) ranged from 0.172–0.298 and within-population H ranged from 0.206 to 0.273 (Figure 4).

Allele number increased significantly ($P < 0.05$) with N_e at the population level (Figure 4). However, the relationship between allele number and N_e was significant ($P < 0.05$) at the subpopulation level only when the outlier, XA-A, was removed from the analyses. The mean allelic diversity over eight polymorphic loci (H) varied independently of the effective size (N_e) of the population or subpopulation (Figure 4).

Discussion

Variation within subpopulations

Most of the total diversity (*c.* 90%) in both allozymes and quantitative characters is explained by variation within subpopulations. Within-family diversity accounts for 62–71% of the total diversity, but between-family diversity is also high (18–28%).

Inspection of subpopulation maps shows that the less common alleles are patchily distributed in space (H.C. Prentice, unpubl.). This aggregation of alleles may be explained by the uneven distribution of plants, with disjunct clumps of intermingled individuals isolated from their nearest neighbours by tens of metres (Prentice 1976, 1984). The spatial clumping of individuals is a consequence of restricted seed dispersal by ants (J. Nebot & I. Mateu, unpublished data). Short-distance pollen dispersal, particularly by solitary bees (J. Nebot, pers. comm.) will reinforce the effect of restricted seed dispersal. Restricted gene flow in obligately outbreeding species has been demonstrated by, for example, Schaal (1980).

Variation between subpopulations and populations

Although subpopulations within the *S. diclinis* populations are usually separated by up to 1 km, a relatively low proportion (4–5%) of the total allozyme diversity and quantitative genetic variation is explained by differences between subpopulations. The between-population component of diversity is also relatively low (5–7%), despite the geographic separation of the populations (Figure 2).

Analysis of the pattern of between-subpopulation differentiation reveals a mosaic of variation in both allele frequencies and in quantitative characters. Subpopulations from the same population are seldom grouped together, even at the higher levels in the dendrograms (Figure 3), although there is a tendency for the western and eastern groups of subpopulations to form high-level clusters in the allozyme dendrogram.

There is a geographically patchy distribution of rare alleles (alleles with a frequency of <10% in the total material) among subpopulations and populations (H.C. Prentice, unpubl.). Some of the rare alleles are restricted to one population, whereas others are present in scattered and widely separated subpopulations. Even the less common alleles (with frequencies of 10–20% in the total material) may be absent from some subpopulations. However, because rare alleles make a negligible contribution to the gene diversity statistic H, relatively little of the total allelic diversity is explained by the mosaic distribution of the rare and less common alleles among populations and subpopulations.

Effective population size and allelic variation

The patchy distribution of the rare and less common alleles among subpopulations and populations suggests that random changes in allele frequencies and the loss of alleles have occurred as a result of genetic drift in small and isolated populations (Nei, Maruyama & Chakraborty 1975; Brakefield 1989). The positive relationship between allele counts and the effective size of the population or subpopulation (Figure 4) provides further support for the idea that the structure of allozyme variation in *S. diclinis* has been influenced by genetic drift. In contrast, there is no significant relationship between N_e and allelic diversity (cf. van Treuren *et al.* 1991).

These results are consistent with the theoretical prediction that the loss of gene diversity will be slower than the loss of alleles in a population which has undergone a rapid reduction in size (Maruyama & Fuerst 1985). The extent of the reduction in gene diversity during an episode of small population size is determined both by the severity of the population bottleneck and by the subsequent rate of population recovery. However, although gene diversity may be rapidly regained in a numerically increasing population, even after a serious reduction in population size, the number of alleles per locus will be more severely affected by genetic drift during population bottlenecks (Nei, Maruyama & Chakraborty 1975).

Because large populations may have lost genetic variation as a result of historical bottlenecks, it may be unrealistic to expect a clear relationship between allelic variation and present-day population size (cf. Lönn & Prentice 1995). The fact that there is a significant relationship between population size and allele number in *S. diclinis* may be related to local habitat availability. The populations that are numerically large at present occur in areas where there is a relatively extensive mosaic of potentially suitable, semi-natural, early successional habitats. These populations are likely to have been relatively large throughout a long historical period. Alleles may be lost, via genetic drift, from a subset of the subpopulations but are retained within the population as a whole. In contrast, the smallest populations are confined to restricted islands of semi-natural habitat within an intensively cultivated landscape, suggesting that they have had a long history of isolation.

Implications for conservation

Use of allozyme or molecular markers to assess genetic variation in conservation-oriented studies makes the implicit assumption that such markers are representative of ecologically important genes, including those that influence life history, physiology and morphology (cf. Ennos *et al.* this volume). In *S. diclinis*, allozyme and quantitative characters show similar partitions of diversity (cf. Schwaegerle, Garbutt & Bazzaz 1986; Prentice 1992), although the details of the geographic pattern detected in the two types of data are different. These findings suggest that the *partitioning* of allozyme diversity may provide a valuable, general overview of the hierarchic structure of genetic diversity within species, but that it may be more difficult to use geographic *patterns* of allozyme differentiation as a basis for generalisation about patterns of variation in other types of character.

Investigation of the structure of genetic variation in particular rare species may help to suggest specific conservation strategies that aim to minimise the loss of genetic variation from populations of that species (Kay & John this volume). In *S. diclinis*, most of the total genetic diversity is stored within populations and little diversity is explained by differences between the spatially isolated populations. However, although the majority of the genetic diversity in the species is stored within populations, different populations contain different combinations of rare alleles, and the rare and less common alleles are patchily distributed between and within subpopulations. The disappearance of a single subpopulation is likely to lead to the loss of several alleles from a population and, in some cases, from the entire species (H.C. Prentice, unpubl.). A conservation strategy that does not give priority to the maintenance of the different subpopulations at each site will lead to the loss of genetic variation in *S. diclinis*.

Studies of genetic structure in rare species may also contribute to generalisations about the relationship between levels of genetic diversity and factors such as distributional area, breeding system or reproductive biology (Hamrick & Godt 1989; Hamrick *et al.* 1991). Such generalisations may be of help to conservation managers who are working with rare species for which no genetic data are available. Information on genetic structure may, in some cases, provide information on the future evolutionary potential of a rare species, or help to point to genetic problems such as inbreeding depression.

Many rare plant species, including *S. diclinis*, are probably more immediately threatened by habitat loss than by evolutionary restrictions imposed by loss of genetic variation (cf. Lande 1988). However, the study of genetic variation in rare species has, in recent times, mostly been motivated in terms of conservation biology or conservation genetics (e.g. Falk & Holsinger 1991).

We would like to emphasise that conservation genetics is not a discipline that can exist in isolation from the mainstream of genetics or population biology. Studies of genetic variation in rare species contribute to our general understanding of the processes that are involved in determining the levels and structuring of genetic variation in natural populations.

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Which measures of genetic variation are relevant in plant conservation? A case study of *Primula scotica*

R.A. Ennos, N.R. Cowie, C.J. Legg and C. Sydes

Ennos, R.A., Cowie, N.R., Legg, C.J., & Sydes, C. 1997. Which measures of genetic variation are relevant in plant conservation? A case study of *Primula scotica*. *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, 73–79. Peterborough, JNCC.

Genetic methods are important in conservation for measuring both the evolutionary potential of populations and the extent and patterns of adaptive differentiation among them. Genetic markers such as isozymes offer a quick means of analysing selectively-neutral genetic variation within and among populations. However, there may be a poor correlation between neutral marker variation and levels of quantitative variation for adaptively important traits. Furthermore the pattern of differentiation for neutral markers is not expected to correspond with that for quantitative traits which are under selection. Caution is therefore required in the interpretation of data obtained from genetic markers when used to draw conclusions about conservation issues.

These difficulties are illustrated by reference to parallel studies of genetic marker and quantitative genetic variation in the Scottish endemic *Primula scotica*. Levels of genetic marker variation are very low in *P. scotica*, but highly significant quantitative genetic variation is found both within and between populations. It is argued that measures of quantitative variation for appropriate traits are more relevant for estimating the evolvability of populations and their degree of adaptive differentiation than are studies based on genetic markers. Where possible, studies of quantitative genetic variation should be included in conservation programmes for threatened species.

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Introduction

Since the emergence of conservation biology as a discipline, much debate has taken place concerning the deleterious long-term genetic consequences of small population size, especially its effects on genetic diversity. Concern is justified because as population size decreases, there is a decline in the equilibrium level of genetic variation that can be maintained by mutation and/or selection in the face of genetic drift (Ellstrand & Elam 1993). This has important implications because the ability of a population to respond to selection is directly related to the level of genetic variation for relevant adaptive characters. In chronically small populations, lack of additive genetic variation for appropriate characters may prevent adaptive responses to alterations in abiotic or biotic environmental conditions, and predispose the population to extinction.

One of the practical roles that genetics can play in conservation is the provision of simple techniques for assessing the evolutionary potential of threatened populations and hence their vulnerability to extinction following environmental change. These techniques should ideally yield quantitative measures of genetic variation affecting characters likely to be important in future adaptation. Two fundamentally different strategies have been used to address this problem. The first involves the direct measurement of levels of additive genetic variation for quantitative characters likely to be involved in future adaptation (Lawrence 1984). The second is an indirect technique, the assessment of variation for genetic markers (isozymes, DNA variants) whose effects on the phenotype are unknown (Schaal, Leverich & Rogstad 1991; Kay & John this volume; Prentice & Andersson this volume). The assumption underlying the use of the indirect technique is that the levels of genetic variation for genetic markers are well correlated with the levels of variation for variable loci affecting traits of future adaptive importance.

In this paper we will argue that there are good theoretical reasons for believing that the levels and distribution of genetic variation estimated by direct (quantitative genetic variation) and indirect (genetic marker variation) methods will not always be concordant (Giles 1983). This implies that we should be very cautious in making inferences about the adaptive potential of populations on the basis of genetic marker data alone. We will illustrate these points by contrasting direct and indirect assessments of the adaptive potential of the Scottish endemic species *Primula scotica* Hook. and discussing the implications of these results for conservation policy.

Measuring adaptive potential

Direct measurement of the adaptive potential of populations involves quantitative genetic analysis of adaptive traits (Lawrence 1984; Mitchell-Olds & Rutledge 1987). Related groups of individuals (populations, families) are grown under uniform conditions. Plants are measured for traits which, in the opinion of the investigators, may be important in future adaptation. Because the experiment is attempting to assess the ability of plants to adapt to environmental change, it is arguably most appropriate to make measurements under novel environmental conditions close to those encountered following environmental change (if these can be anticipated). Phenotypic variation among individuals is partitioned among and within populations and families by analysis of variance, and the extent of genetic variation among and within populations is quantified. The genetic variation detected is assumed to be caused by differences at many unidentified genetic loci affecting the trait concerned.

Indirect measurement of the adaptive potential of populations exploits the rapidly developing suite of techniques (isozymes, RFLP, RAPD, etc.) that allow screening of variation in the genetic information possessed by plants (Burke, Rainey & White 1992). The beauty of these techniques, which accounts for their widespread application in conservation, is that they yield measures of genetic variation and its distribution that are largely unaffected by environmental conditions. Analysis can be conducted on small samples from the wild without the need for prolonged culturing of plants. However, the nature of the variation detected is very different from that detected by quantitative genetic analysis. As genetic marker variation is generally assumed to have negligible effects on both plant phenotype and fitness (Avise 1994), the analysis gives quantitative estimates of selectively neutral variation for a sample of single loci.

Discrepancies between measurements

There are two reasons why measures of genetic variation and its distribution derived by the two methods outlined above may be discordant. The first is because quantitative genetic analysis is based on measures of variation in continuously varying or metric traits influenced by many genes whereas genetic marker analysis is based on measures of variation at single loci (Lande & Barrowclough 1987). Suppose that a population has passed through a bottleneck and that allelic diversity has been reduced to zero. The restoration of allelic variation at a particular marker locus will be a very slow process, relying on the generation of new alleles by mutation at a rate of roughly 10^{-5} per generation at isozyme loci, for instance. Populations may remain monomorphic at this marker locus for many hundreds or thousands of generations. The situation is different for adaptive traits. Here mutations at many different loci may potentially contribute to the regeneration of quantitative variation. Although the probability of any one of these loci mutating will be the same as for the single locus case, the combined probability that at least some of this number will mutate and regenerate genetic variance is high. Thus the restoration of quantitative genetic variation for adaptive traits will be much swifter after a bottleneck than will be the restoration of genetic diversity at individual marker loci. Indirect analysis using genetic markers will thus give much lower estimates of genetic diversity following a bottleneck than will direct estimates of quantitative genetic variability for adaptive traits. From the point of view of conservation the direct estimate of diversity will be the relevant one, because it reflects the evolutionary potential of the population.

The second reason for anticipating discordance between estimates of genetic diversity is that the two types of genetic variation measured have different impacts on plant fitness. Most genetic markers are assumed to be selectively neutral (Avise 1994). The distribution of genetic marker variation within and among populations (genetic structure) is governed by a balance between drift and migration, together with some historical effects. There is no relationship between the spatial pattern of genetic variation and underlying environmental variability. In contrast genetic structure for alleles underlying adaptive character variation will be affected not only by by drift, migration and history, but also by natural selection. If selective differences are strong among sites, selection will be the dominant factor structuring the variation, and there will be a correlation between the spatial distribution of genetically determined trait variation and underlying environmental variation (Jain & Bradshaw 1966). The genetic structure for adaptive traits and genetic markers will thus be very different. Studies of genetic markers may not reveal important adaptive genetic differences between populations which need to be taken into account in the development of conservation policy.

The conclusions to be drawn from this brief consideration of theory are twofold. The first is that there are important circumstances in which quantitative genetic analysis and genetic marker analysis of the same plant populations are likely to yield different results with respect to levels of genetic diversity and its distribution. The second is that in both cases it is the measures of quantitative genetic variation which are of more relevance to conservation than results from genetic markers. In order to demonstrate that these conclusions have practical relevance, it is instructive to describe some genetic aspects of an ongoing conservation study of the Scottish primrose *Primula scotica.*

Genetic variation in *Primula scotica*

Primula scotica is a small but distinctive perennial that grows typically in the short turf at the tops of sea cliffs, as well as in calcareous flushes and on more inland dune grassland sites (Figure 1) (Bullard *et al.* 1987). The species is an allohexaploid related to the diploid *P. farinosa* and the octoploid

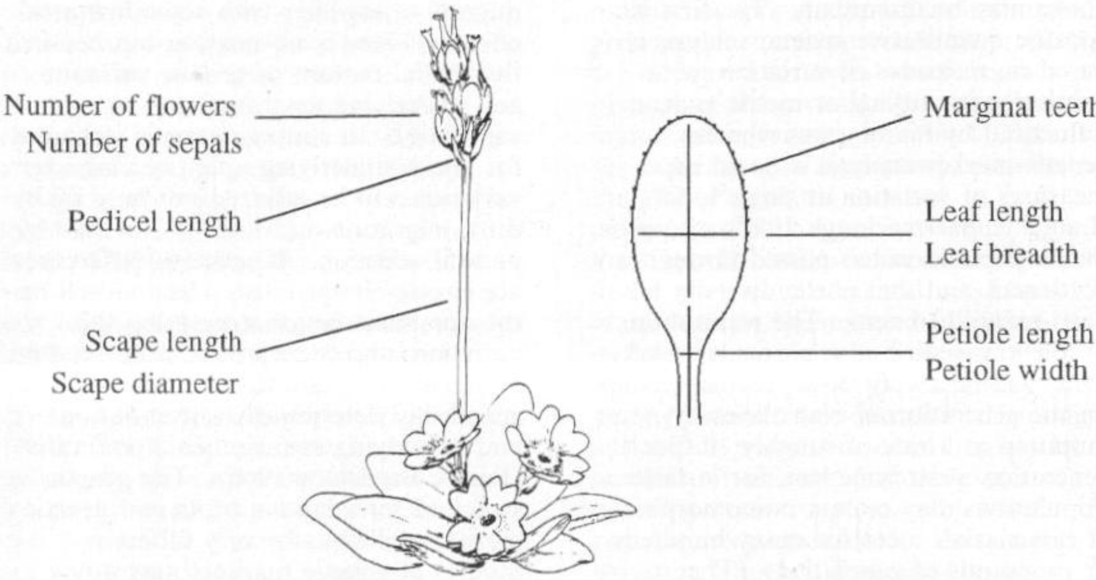

Figure 1. *Primula scotica* showing both long and short scapes, and the characters used in quantitative analyses.

P. scandinavica. Flowers are homomorphic, and the plant is believed to be predominantly self-pollinating (Mazer & Hultgard 1993; Tremayne & Richards this volume).

Primula scotica is one of the few endemic plants in Britain, its world distribution being restricted to Orkney and scattered populations along the north and east coasts of Sutherland and Caithness (Figure 2.). Of the 30 populations recorded on Orkney last century, only 15 are now known to remain (Berry 1985; Scott 1990).

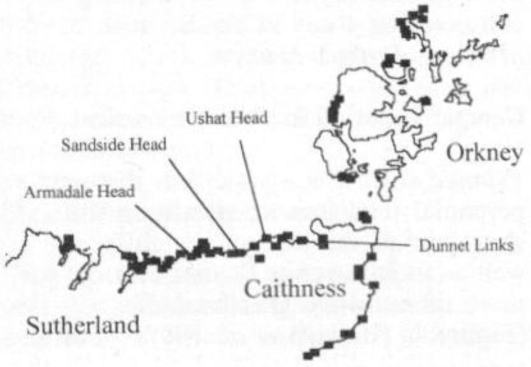

Figure 2. Map of distribution of *Primula scotica* in Sutherland, Caithness and Orkney.

Although not locally rare, the plant carries high conservation value as an endemic with a restricted world distribution and clear threats of local extinction at some of its remaining sites. It is also pertinent to note that the distribution lies at the extreme northern limit of the British Isles. This means that in the event of global warming the species does not have the option of migrating north, and continued existence must rely on adaptation of the populations to a changed environment *in situ*.

Under these circumstances it is important to obtain some idea of the adaptive potential of the *P. scotica* populations. Do they contain sufficient genetic variation to allow a genetic response to climatic change? Furthermore, we would also like to know the extent of genetic differences between populations so as to guide conservation policy in the event of threats to the continued existence of the populations. How many populations need to be conserved to include the full range of genetic variation, and where are these sited? If an *ex-situ* conservation programme were found to be necessary, what sampling strategy should be adopted to sample the gene pool efficiently?

Two contrasting research programmes have been carried out independently to estimate genetic variation in *P. scotica.* The first used genetic markers to obtain an indirect estimate of the adaptability of populations and the distribution of genetic variation (Glover & Abbott 1995). Variation was scored in plants from 14 populations at 15 putative isozyme loci, and RAPD analysis was conducted on a total of 14 plants from four populations using six primers.

RAPD analysis indicated no differences in banding patterns among the plants scored. Isozyme analysis revealed segregation at only one of the 15 loci. However, it demonstrated 'fixed heterozygosity' but no segregation at a high proportion of the remaining loci. This result is consistent with a single allopolyploid origin for *P. scotica*, the alternate alleles in the 'fixed heterozygotes' presumably coming from different parents (Crawford 1989). The polyploid origin of the species means that genetic diversity analysis normally used in diploid species cannot be applied directly. Substantial allelic diversity is found in these populations, but because of their polyploid origin the diversity at isozyme loci is predominantly within individuals. The segregation of this variability is clearly dependent on the pairing behaviour of chromosomes from different parents at meiosis. Technical difficulties mean that variation in the dosage of particular alleles, which would be expected in a segregating population, may not be apparent.

Under these circumstances it is difficult to make inferences about the adaptive potential of the population from genetic marker data alone. Moreover the potential lack of correlation between genetic marker variation and adaptive potential should make us cautious about over-interpretation of information on genetic markers. Despite these difficulties Glover & Abbott (1995) drew a number of far-reaching conclusions from their work which they used to make recommendations about conservation policy. The first of these was that *P. scotica* has a narrow genetic base, and is unlikely to be able to respond to habitat changes. The second is that there would seem to be no real genetic differences between populations from different sites. In order to test these inferences and conclusions we initiated a second study to analyse quantitative genetic variation in *P. scotica* as a direct measure of its evolutionary potential (Cowie *et al.* unpublished).

Material for the experiment was collected as families of seed from four mainland populations: Dunnet Links, Armadale Head, Ushat Head and Sandside Head. Dunnet Links is located approximately half a kilometre inland on dune grassland sheltered behind the main dune ridge, whereas the remaining populations are from maritime heath and grassland communities on exposed cliff tops. Families were grown in a completely randomised block in the glasshouse for 9 months and measured for the following 11 characters which may be of adaptive significance to the plants (Figure 1).

Leaf length
Leaf width
Petiole length
Petiole width
Number of rosettes
Number of teeth per cm leaf margin
Mean number of sepals
Scape length
Pedicel length
Scape diameter
Number of capsules

Casual inspection of plants in the greenhouse suggested significant differences in morphology among the four populations. To illustrate the extent of differences between populations, data from the 11 characters measured were incorporated in a principal component analysis. Figure 3

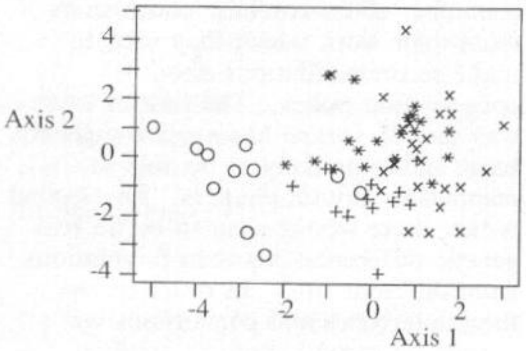

Figure 3. Principal components analysis of quantitative characters for families of plants grown in the glasshouse from seed collected at Dunnet Links (○), Armadale Head (+), Sandside Head (∗) and Ushat Head (×).

shows a plot of the family scores on the first two principal component axes. The inland population from Dunnet Links is distinct from all others on the first principal component axis, whereas Sandside and Armadale are well separated on the second axis.

To quantify the distribution of genetic variation within and among populations, and to obtain measures of the heritability of variation for the measured traits, variation was partitioned into its components by analysis of variance. Variance components were calculated, taking into account the unbalanced sampling scheme, and the percentages of variation attributable to differences between populations, between families within populations, and within families were calculated (Table 1). On average some 35% of variation is attributable to differences between populations, 9% to differences between families within populations, and the remainder to within-family variation. Minimum estimates of the heritability of variation were calculated on the assumption that individuals within families are the products of self-fertilisation (Table 1). Six of the 11 characters showed significant heritability of variation, indicating the potential of populations to respond to selection on these characters.

Table 1. Components of variation in characters of *Primula scotica*. Percentage variation attributable to populations, families and individuals within families for eleven characters measured on plants grown in glasshouse conditions from seed collected from Dunnet Links, Armadale Head, Sandside Head and Ushat Head. h^2 is the estimated heritability for each character. * $0.05 > P > 0.01$; ** $0.01 > P > 0.001$; *** $P < 0.001$.

Character	Populations	Families	Individuals within families	h^2
Leaf length	30	2	68	0.03
Leaf width	53	11	36	0.22 ***
Petiole width	22	18	60	0.23 ***
Petiole length	46	2	52	0.03
No. rosettes	34	5	61	0.07
No. teeth	29	11	60	0.16 *
No. sepals	37	13	50	0.2 **
Scape length	51	6	43	0.1 *
Pedicel length	36	6	58	0.13
Scape diameter	42	4	54	0.08
No. capsules	5	24	71	0.25 ***

We can conclude from this experiment that far from having a narrow genetic base, *P. scotica* is a genetically variable species. A large part of this variation is distributed among populations, but there is also significant genetic variation within populations, at least for some characters. From the point of view of conservation, *P. scotica* may well be able to respond to environmental change *in situ*, though this does not of course mean that it is not threatened by the manifold effects of climate change. The large genetic differences between populations imply that a range of sites needs to be conserved if the gene pool of the species is to be maintained *in situ*. Likewise if *ex-situ* conservation is necessary, sampling needs to be carried out from a full range of sites where contrasting environmental conditions prevail.

Conclusions

Although we have concentrated our attention in this paper on one particular example, the results that are presented here have wider messages for plant conservation genetics. The principal one is that we must not rely too heavily on the use of genetic markers as a means of assessing the adaptive potential of threatened plants, or of the distribution of genetic variation among their populations. We need to be aware of the limitations of the techniques which we are using and the reliability of inferences that can be drawn from the data obtained (Ennos 1995). As the example of *P. scotica* shows, the over-interpretation of genetic marker data can lead to erroneous conclusions being drawn about the genetic variability of plant species, and seriously inappropriate guidelines for conservation being drawn up.

The second message which we would like to convey is that quantitative genetic analysis of plant variation has much to offer conservation. In many ways this type of analysis is far more suitable than genetic marker analysis for answering questions of conservation interest. For small and short-lived plants the resources demanded are relatively modest, and the low technology approach, while it may not be appealing to grant-awarding bodies, may mean that meaningful conservation genetic studies can be undertaken even where resources are scarce.

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Use of molecular genetics in the formulation of insect conservation strategies

Andrew S. Pullin, Janet Cameron and Christina H. James

Pullin, A.S., Cameron, J., & James, C.H. 1997. Use of molecular genetics in the formulation of insect conservation strategies. *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, 80–86. Peterborough, JNCC.

This paper presents some of the problems and challenges in conserving insect diversity and argues that molecular genetics can provide valuable information which helps in resolving problems and formulating conservation strategies. Molecular genetics is also important in providing a genetic component to measuring biodiversity and placing conservation within an evolutionary framework. Four common questions faced in formulating conservation strategies are posed. 1. How do we prioritise protection for species fragmented into small populations throughout their range? 2. Is there gene flow between fragmented populations? 3. Which donor population(s) should we use in a species restoration programme? 4. To what extent is genetic diversity regionalised within a biotope? Examples are given to illustrate these and data are presented which help to resolve the type 1. question raised by the *Aricia* species complex in the UK. Allozyme analysis of genetic distance suggests that the current taxonomy of this complex should be reviewed, particularly the use of voltinism for distinguishing between *A. agestis* and *A. artaxerxes*.

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Insect biodiversity

Over 50% of currently named species are insects and this proportion is likely to rise as more species are described (World Conservation Monitoring Centre 1992). The battle to conserve biodiversity is therefore heavily dependent on our ability to conserve insects (Wilson 1993), but they are still largely understudied in conservation terms. Entomology is a well developed discipline with a large body of literature to support it, yet the vast majority of work has been carried out on common pest species. The insect conservation literature is relatively new and relies heavily on work on butterflies (New *et al.* 1995), and particularly butterflies in the UK (Thomas 1991; Dennis 1992; Pullin 1995).

The recognition that biodiversity can be viewed at three scales, genetic, species and community, has emphasised the inequality of our knowledge in favour of the latter two. Molecular genetics offers opportunities to study diversity at the genetic level and therefore begin to redress the balance. The aim of this paper is to present some of the problems and challenges we face in trying to conserve insect biodiversity, using the UK fauna as an example, and to illustrate the contribution that molecular biology can make to our understanding of the distribution of biodiversity and the formulation of strategies which will be required to conserve it in the future.

Origins and patterns of insect biodiversity

The UK insect fauna is relatively impoverished when compared with continental Europe due firstly to recent glacial incursions forcing repeated colonisations, and secondly to our post-glacial isolation due to rising sea levels (Dennis 1992). The patterns of species and genetic diversity we see today are very recent in origin, largely resulting from colonisations after the last glaciation around 10,000 yrs BP. The distribution of species prior to human influence, some 5000 yrs BP, would have therefore depended on a combination of colonisation ability, persistence and the opportunity provided by the dynamics of habitat availability.

The genetic diversity within a population is the result of the original founding gene pool and subsequent levels of emigration and immigration, plus the ongoing effects of genetic drift and selection. For highly mobile species, the original pattern of colonisation may have been obscured by subsequent gene flow and more recent changes in distribution. For highly sedentary species the pattern may have been fragmented by the large-scale habitat changes caused by human activity. The implications for the patterns of genetic diversity within species and communities are profound. In recently founded populations of relatively sedentary species the founding gene pool will be particularly important, and in small populations chance events will lead to further differentiation among populations giving rise to what Avise (1994) has termed a historical biogeography. Increasing human impact in terms of fragmentation, isolation and reduction in size of populations has probably accentuated this process of differentiation and structuring of species and genetic diversity (e.g. Dempster 1991). In short, the genetic diversity within species is unlikely to be distributed uniformly among populations except in highly mobile panmictic species (Shreeve, Dennis & Pullin 1996). The genetic diversity within populations of sedentary species may reflect local adaptation as well as genetic drift (see Brakefield 1991). At present we do not know how important the maintenance of interpopulation diversity is to long-term species survival.

Problems arising for insect conservation strategy

Human activity has fundamentally influenced the distribution of insect biodiversity. Natural habitats have been changed to form semi-natural ones maintained by grazing, mowing or other repeated disturbance. These areas have now been further reduced in size and are being increasingly fragmented to the point where populations become vulnerable due to their small size and degree of isolation.

The response to this problem has been to protect remaining fragments and try to manage them sympathetically to retain what is left. Although many failures have occurred (Thomas 1991), in particular species have been lost from small nature reserves, this strategy has largely been successful in achieving the short-term aims of conserving examples of high biodiversity semi-natural habitats and many of the rare species that they support. However, we have seen from the previous section that in the past the system we are dealing with has been very dynamic. Communities change over time in response to environmental conditions and species and populations change in genetic make-up over time as they respond to their environment. We can be sure that environmental conditions will continue to change, probably faster than in the recent past, and just as importantly patterns of evolution will change as a direct result of human activity. Our conservation strategy will therefore have to change.

It is often overlooked in conservation that to protect a population in a nature reserve does not protect the species, even if that population is large and secure in the long term (see Rojas 1992). This strategy only conserves a proportion of the genetic diversity that the species carries at that point in its evolutionary history. All other geographical variance may be lost, together with capacity for gene flow between populations with consequent loss of adaptability and resilience to change. We would therefore argue that the strategies that have been used in conservation have not been species conservation strategies. We have been attempting to conserve small fragments of species gene pools, many of which are doomed to extinction in the long term, and in some cases, promoting the speciation process by ignoring the necessity for gene flow in maintaining species integrity. This is particularly the case in some taxa, such as insects, which contain many sedentary species whose populations easily become isolated.

The realisation of such problems has recently led to a shift in emphasis to management on larger scales, landscape ecology and consideration of 'natural areas' (English Nature 1993). In the context of the UK landscape this means more sympathetic management of the wider countryside and particularly agricultural land. It also increasingly means restoration of degraded habitats in an attempt to reconnect populations. Both of these are expensive tasks and require careful decision making and soundly based strategies. We believe that the best way to formulate these strategies is to use an interdisciplinary approach which combines information from many scales ranging from the landscape to genetics.

Some common conservation questions

In order to help visualise the possible contribution of genetics in this context, we present below four questions arising in formulating conservation strategies, together with insect examples which are currently being studied in our laboratory. The first three relate to species conservation and the fourth to communities. We believe that molecular genetics can make a significant contribution to answering these questions and therefore formulating appropriate conservation strategies.

1. How do we prioritise protection for species fragmented into small populations throughout their range?

Most UK species have already experienced recent fragmentation and, for the reasons discussed above, the distribution of genetic diversity among the remaining populations will not be uniform. Nor will the resources for their protection be unlimited. Almost inevitably some will have to be afforded more protection than others. This problem becomes particularly important when taxonomic status is unclear. How can this decision be made so that maximum genetic diversity is maintained?

Example: The genus *Aricia* is currently thought to be represented in the UK by two species, the brown argus *A. agestis* and the northern brown argus *A. artaxerxes* (the latter being separated into two subspecies, *artaxerxes* and *salmacis*). The species are very similar in morphology, although there are a number of supposedly consistent differences, and they are usually separated on the grounds that the former is bivoltine and the latter univoltine. This difference is not usually used to separate species and many 'good' species contain both univoltine and bivoltine populations, including the closely related common blue *Polyommatus icarus*. Importantly, Kudrna (1986) separates northern UK *A. artaxerxes* from northern European populations which he refers to as *A. allous*. Both putative British species of *Aricia* are declining and if *A. artaxerxes* is a true species, it is our only endemic butterfly and should be a very high priority for protection.

2. Is there gene flow between fragmented populations?

The importance of single population protection increases when that population is isolated from all others because if a species exists in some form of metapopulation with regular gene flow, extinction can be counterbalanced by colonisation so long as the habitats remain suitable. How can we judge how important it is to prevent extinction in individual populations?

Example: The British subspecies of the swallowtail butterfly, *Papilio machaon britannicus*, is confined to a number of definable populations in the Norfolk and Suffolk Broads. Some of these are remote but the species is quite mobile. The populations are centred around the river valleys of the Bure, Ant, Thurne and Yare and movement between them would require crossing of unsuitable habitat. Is this a metapopulation with regular gene flow or a number of isolated populations?

3. Which donor population(s) should we use in a species restoration programme?

Following the decline of many of our insect species there are numerous programmes for their restoration into former habitats. Selection of an appropriate donor population is a key component of a properly planned programme. In recent years donor selection has been made on ecological grounds, selecting the population whose ecology most closely matches the population formerly inhabiting the target area. But in some cases the choice is not clear and molecular genetics may be able to add significant evidence to help in decision making.

Example: The last English populations of the chequered skipper butterfly *Carterocephalus palaemon* went extinct in the 1970s. A small group of populations survives in western Scotland and the species is widespread but local on the continent. The cause of the English extinction is thought to have been the decline in appropriate woodland management over its former English range, with the decline in coppicing leading to a closed canopy being a key factor (Collier 1986). This situation has been reversed in some of its former sites and a re-establishment programme is being undertaken. The choice of donor population was not easy because, although populations still exist in Scotland, the ecology and habitat requirements of these northern populations seem to be different from the former English ones. The latter seem to have had similar habitat requirements to populations in Belgium and northern France (Ravenscroft & Warren 1992). The decision to bring donor stock from the continent rather than use Scottish stock is controversial and it is helpful to assess whether the ecological differences between Scottish and Continental populations are underpinned by genetic differences.

4. To what extent is genetic diversity regionalised within a biotope?

Patterns of post glacial colonisation may be species-specific, but may also be closely linked to habitat. Many of our insects are confined to particular biotopes and characteristic groups of species can be found wherever that biotope occurs. The rapid loss of some of our species-rich biotopes, such as fenland and calcareous grassland, has been a centre of conservation concern and has led to the formation of a patchwork of nature reserves and other protected sites. This process of protection will continue, but resource limitation dictates that an overall plan for each biotope or representative community is required. The advent of the natural areas scheme (English Nature 1993) highlights this need. The biodiversity in one natural area is likely to be different from another, but how different? Additionally, the pattern of protected areas is not entirely representative. We do not know how much of our genetic diversity is represented in protected areas. This can obviously be viewed from a species or community perspective.

Example: Calcareous grassland supports one of our most diverse insect faunas and representative areas are scattered across the UK. We have some idea of the distribution of species among calcareous grassland regions, but no idea of the distribution of genetic diversity within species and within and among regions. At the extremes, all regions could contain the same genetic diversity for a given species, or each region could have completely unique diversity. Also the pattern could be common to many species or unique to each species. Patterns and priorities for strategies designed to conserve biodiversity urgently need this kind of genetic information.

An example based on preliminary data

As an example of our approach, we present preliminary results of investigation into the *Aricia* species complex mentioned above.

Allozyme analysis was chosen as a method to measure genetic diversity within and relatedness among populations within the *Aricia* species complex in the UK. A total of 183 individuals from eight populations were sampled during 1994 and 1995 (Figure 1). Two of the chosen sites represent typical populations: *A. agestis* from Dorset (1) and *A. artaxerxes artaxerxes* from Galashiels, Borders, (8) following the taxonomy of Emmet & Heath (1990). The remaining six were chosen by reference to previous work (Frydenberg & Hoegh-Guldberg 1966; Hoegh-Guldberg & Jarvis 1970) to represent the transition between populations recognised as *A. agestis* (4) to populations recognised as *A. artaxerxes salmacis* (7). Sample sizes ranged between 14 and 35 (Table 1), the lower figures being imposed by the respective reserve managers due to the small size and vulnerability of the populations.

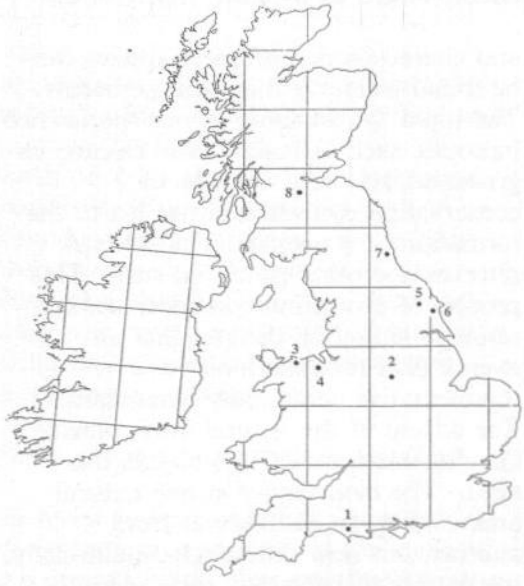

Figure 1. Map of sites from which samples of *Aricia* populations were taken: 1 Swanage, Dorset; 2 Lathkilldale, Derbyshire; 3 Bryn Meriadoc, Clwyd; 4 Eryrys, Clwyd; 5 Gundale, North Yorkshire; 6 Fordon Bank, North Yorkshire; 7 Durham; 8 Galashiels, Borders.

Table 1. *Aricia* collection sites, sample sizes, current taxonomic status and voltinism for populations assessed by allozyme electrophoresis.

Collection sites	Sample size	Taxonomic status	Voltinism
1. Swanage	27	*A. agestis*	2
2. Lathkilldale	35	*A. artaxerxes salmacis*	1
3. Bryn Meriadoc	14	?	2
4. Eryrys, Clwyd	17	?	1
5. Fordon Bank	20	?	2
6. Gundale	23	?	1
7. Durham	28	*A. artaxerxes salmacis*	1
8. Galashiels	21	*A. artaxerxes*	1
9. Outgroup: Keele	14	*Lycaena phlaeas*	

Nei's genetic distances (Nei 1978) were calculated from genotype designations using Genestrut (Constant 1994) and are shown in Table 2. A neighbour-joining tree (Saitou & Nei 1987) was constructed using these distances to visualise population relationships (Figure 2).

These provisional results show that the populations are not clustered according to voltinism nor according to currently accepted taxonomy, except for the isolation of the *A. artaxerxes artaxerxes* Galashiels population from the rest.

Table 2. Nei's unbiased genetic distance (D) calculated using GeneStrut (Constant 1994) from eight polymorphic loci comparing populations of *Aricia* and using small copper *Lycaena phlaeas* as an outgroup.

Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1. Swanage	–								
2. Lathkilldale	0.183	–							
3. Bryn Meriadoc	0.229	0.156	–						
4. Eryrys	0.082	0.325	0.243	–					
5. Fordon Bank	0.323	0.204	0.121	0.393	–				
6. Gundale	0.357	0.256	0.210	0.411	0.034	–			
7. Durham	0.200	0.034	0.140	0.338	0.111	0.160	–		
8. Galashiels	0.238	0.147	0.188	0.221	0.209	0.238	0.123	–	
9. Small copper	0.453	0.315	0.397	0.483	0.545	0.630	0.339	0.170	–

The populations are clustered according to geographical location except for the Welsh populations, one of which is grouped with the *A. agestis* population from Swanage, and the other with the northern England populations. Importantly, the relative genetic distances involved suggest more of a distinction between *A. artaxerxes artaxerxes* and the rest than between *A. artaxerxes salmacis* and *A. agestis*. On the basis of these results we suggest that there is insufficient evidence to support the notion that two distinct species exist in the UK, and that if they do, it is unlikely to be in agreement with currently accepted species boundaries.

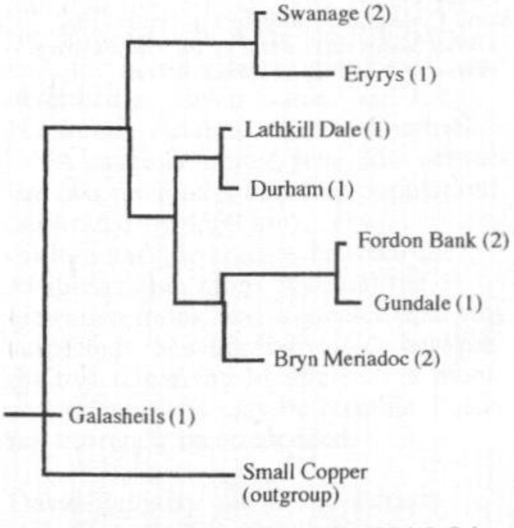

Figure 2. A neighbour-joining tree (Nei & Saitou 1987) indicating genetic similarity between eight *Aricia* populations and an outgroup (small copper *Lycaena phlaeas*). The number of generations characteristic of each population is shown in brackets.

Clearly more work needs to be done, particularly focusing on additional populations within the transition zone, and using other, independent, molecular methods of analysis before any firm conclusions can be reached. At this stage we would not recommend increasing the priority afforded to northern populations of *Aricia* on the grounds that they represent our only endemic species of butterfly. However, more importantly, we would recommend that populations throughout the UK should be protected to preserve the variation that is evident.

The message to conservation organisations is that the use of molecular genetics in conservation is in its infancy and most of the studies in which it has been applied are currently incomplete. But the potential for its future contribution to formulating the strategies for conserving the genetic component of biodiversity cannot be doubted.

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