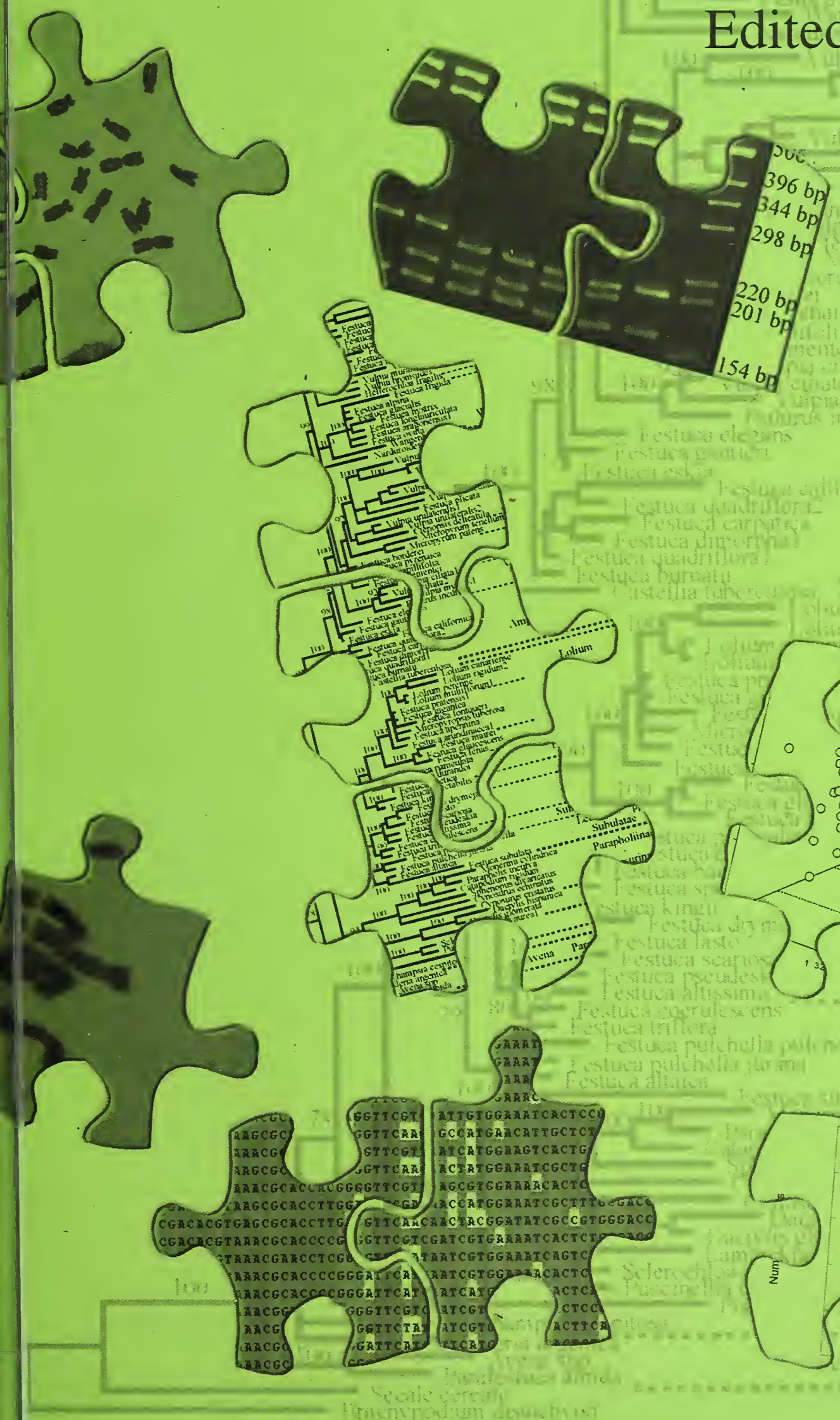
CURRENT TAXONOMIC RESEARCH ON THE BRITISH & EUROPEAN FLORA

Edited by J.P. Bailey
R.G. Ellis



BOTANICAL SOCIETY OF THE BRITISH ISLES
Conference Report No. 25

CURRENT TAXONOMIC RESEARCH ON THE BRITISH & EUROPEAN FLORA

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ON THE

BRITISH & EUROPEAN FLORA

Proceedings of a Conference arranged by the Botanical Society of the British Isles to commemorate the retirement of Professor Clive Stace from the University of Leicester

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13th -14th September 2003

Edited by

John Bailey and Gwynn Ellis

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FRONTISPIECE

Clive prepares to cut the cake after the conference meal

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Preface

DR JOHN P. BAILEY

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This volume contains papers given at the 'Current Taxonomic Research Work on the British & European Flora', meeting held in Leicester on 13-14 September 2003. This meeting was organised by the BSBI to commemorate the retirement of Clive Stace from the University of Leicester. As befits a meeting celebrating the considerable contribution to British botany by Clive Stace the papers closely mirror his particular interests, but still form a cohesive whole in the area of Floristics and Phylogeny. All the contributions are by professional botanists, three of them having gained their PhD's at Leicester. Papers range from the Floras of particular regions, through new approaches to Keys and electronic Floras through to more detailed studies of particular plant groups. The intelligent application of DNA based technologies to plant phylogenetics and population studies has greatly increased our understanding of apomict groups, evolution of new taxa and the classification of certain families. The taxonomy of northern terrestrial Orchids, for instance, has been drastically redrawn eliminating many former inter-generic hybrids. Apomicts are also well-represented in this volume with papers on both *Pilosella* and native *Limonium*. This volume takes an optimistic look at the future directions of plant taxonomy and will be of value to anyone interested in the British and European Floras.

Clive Stace was born in 1938 in Tunbridge Wells, where his early interest in natural history was nurtured and encouraged by Miss Aline Grasemann. In 1962 he gained his Ph.D and was appointed as a lecturer in Botany at the University of Manchester. In 1974 he became a Reader in the Botany department at Leicester University maintaining the taxonomic tradition there started by Tom Tutin. In 1985, when personal chairs were conferred much less readily than today, he was awarded a personal chair in Plant Taxonomy. In addition to his busy and inspirational teaching duties at both undergraduate and post-graduate level, he also had two lengthy spells as head of Department.

He joined the BSBI in 1958, and became an editor of *Watsonia* in 1972, rising to senior editor. He was elected President of the Society for 1987 and 1988. He has published dozens of papers, mainly on the Poaceae, the British Flora and the Combretaceae. His book Plant *Taxonomy and Biosystematics* has gone into two editions, whilst *Hybridization and the Flora of the British Isles* is an expensive and much sought after text. His major work, the *New Flora of the British Isles*, was completed in 1991, with his wife Margaret producing camera-ready copy in order to keep production costs down so as to make the volume readily affordable. This has gone into a more comprehensive second edition and also recently appeared in an interactive DVD-ROM form with the *New Atlas* data and a comprehensive collection of images.

The meeting took place in the University accommodation in Oadby, with many of the delegates spending the night there. All speakers invited were happy to give their time, and it gave us particular pleasure to able to bring Franta Krahulec (Czech Republic) to Britain for his first visit. We should not forget the two that 'got away' – Mick Crawley and Ray Woods who both gave stimulating and interesting talks, but who have been unable for one

reason or another to produce papers. Franklyn Perring, despite his most ardent desire to attend right up until the last moment, was sadly defeated by his final illness and Phillip Oswald kindly gave Franklyn's presentation at very short notice. The talks all elicited much questioning and debate, which was carried on into the conference meal, at which Clive was presented with a cake bearing the cover of his Flora printed in the icing.

On the Sunday we had an excursion to Derbyshire, ably led by Roy Smith, Nick Moyes and Alan Willmot, to see such choice plants as *Sesleria caerulea* and *Trientalis europaea*, both at or near their southern limit and restricted to a metre^2 or less! The weather was very kind to us and we had a most enjoyable trip (see Colour Plates 3, 4 & 5). I trust that Clive enjoyed the weekend, surrounded as he was by friends and colleagues from over the years, and wish him and Margaret a long and happy retirement and the leisure to pursue all his interests - new and old!

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What is happening to plant taxonomy?

CLIVE A. STACE

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The meeting held on 13-14th September 2003 gave me enormous pleasure, not only because so many friends and colleagues were present, but also because it brought together an exciting group of botanists covering many topics of the greatest interest. In some ways it was reminiscent of the first 16 conferences of the BSBI (held between 1948 and 1977), which served to summarise existing knowledge and thinking on mainstream plant taxonomy in Britain. This view is admittedly rather self-indulgent, because the speakers in 2003 were suggested by me, but I cannot imagine that all the 100 or so participants were not similarly pleased and fascinated to hear authoritative talks by people respected as leading workers in their fields. It was a rare treat.

I am also delighted to be able to say that the average age of the speakers was significantly below mine, especially as the lack of young taxonomists today is a frequently articulated concern. It is true that many, perhaps most, of the speakers would not call themselves taxonomists, but the fact is that their research concerns the genetic range and diversity of organisms, and that they therefore need to be able to identify taxa accurately. Their subjects are what I would call taxocentric, i.e. taxonomy in the broadest sense.

Nowadays taxocentric research is not considered to be of the highest importance, and it is very poorly funded. There are relatively few jobs to be found in our subject, and young people, who quite rightly strive to achieve gainful employment, are therefore less attracted to it during their courses of education. Only a very small number of Universities now offer modules in plant taxonomy or even in the more general field of plant diversity. It is absolutely true to say that graduates have 'progressed' from not knowing the differences between mosses and liverworts to now not knowing the differences between bryophytes and pteridophytes. Not very important, it is said. Yet such knowledge is more vital than ever, for it is fundamental to topics as diverse as conservation practice (for instance, are *Epipactis youngiana* or *Dactylorhiza lapponica* more or less important than *E. sancta* or *D. ebudensis*?), climate change (know your Conyzas before drawing conclusions), or the leakage of GM crops into the wild (but first become familiar with *Brassica rapa*, *B. napus* and their subspecies and hybrids), to name but three.

Probably the most newsworthy developments in plant taxonomy in recent years are the revelations arising from molecular systematics, i.e. the use of DNA and proteins (now mostly DNA sequencing). When we learn that there seem to be no differences between *Gentianella amarella* and *G. anglica*; or that after all *Nasturtium* is not so closely related to *Rorippa* and should be re-separated; or that *Cardaminopsis* is not a distinct genus, nor even part of *Arabis*, but part of *Arabidopsis*; or that *Linaria* and allies belong not to Scrophulariaceae but to Plantaginaceae, then we realise that there is brewing in plant taxonomy a revolution that will see our subject change rapidly, and which will demand our close attention over the next exciting years. Mercifully there was no paper at our meeting on molecular taxonomy, any more than there was one on microscopy, floral dissection, chromatography or any other

technique, but there were many that could not have been written without its results. Molecular systematics, if it is to make its logical and deserved impact on taxonomy, must be seen as a technique, not a new science or way of life. In my view research needs to be taxon-based, not technique-based. It is essential to gain a deep and wide-based knowledge of a group, preferably including an extensive field study, before new techniques are applied. The attitude 'I have this new technique; to which taxon can I apply it?' is not likely to achieve best results. Or, in the words of Kaplan & Fehrer (*Folia Geobotanica* **39**: 431, 2004), 'long-term taxonomic expertise usually generates very well-founded specific questions suitable for.....appropriate molecular methods'.

The need to capture the interests of the younger generation and to generate a fresh core of taxonomic expertise, including the ability to identify plants, is the subject of several of the speakers, notably Franklyn Perring, Richard Pankhurst and Ruud van der Meijden. Having taught undergraduates for 44 years I am well aware that two of the greatest barriers to acquiring taxonomic expertise are the complex terminology involved, and the bewildering complexity and strange language of diagnostic keys. Ways around these problems are now being actively explored, and I have no doubts at all that in the future our Floras will undergo quite radical transformations, making them more comprehensible and user-friendly, i.e. more likely to produce the right answer. I rate this along with molecular systematics as the most important current developments in plant taxonomy.

Finally, I would like to pay tribute to our fellow taxonomists in other European countries. For too long many have arrogantly thought that in Britain we do it best and know it most completely, without any objective justification. As anyone who has made botanical trips abroad knows full well, there is an enormous amount that we can learn from our Continental colleagues, many of whom are at the cutting edge of our science and have developed a profound knowledge of their floras. I am constantly humbled by my ignorance when visiting them. It was particularly appropriate, therefore, that four of our speakers came from countries (Sweden, Czech Republic, The Netherlands, Spain) about which the above is especially true. I and the rest of the audience owe a debt of gratitude to all the speakers for their time and effort in making this such a stimulating meeting, and to John Bailey for organising it all.

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Keys for the future

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ABSTRACT

Diagnostic keys for the identification of plants have been in use for hundreds of years, but computerised keys have only been around for the past thirty. The internet provides a handy means to publish keys, but the really important innovation lies in programs for interactive multi-access identification. There are hundreds of these available, but computerised descriptions of taxa in the British flora are scarce. Data exists for particular groups, such as *Carex* and *Taraxacum*, but the CDROM being prepared by ETI is planned to include a key for the whole flora. Although the complete list of different characters will be long, the number of characters needed for descriptions of individual taxa will be manageable. Published Floras, admirable though they are, are intentionally concise and severely limited in the amount of information that they can contain. In a computerised flora, there is no reason to not include complete descriptions, not to mention drawings, pictures, and maps . This data will need to be organised in some kind of database. It is not clear whether we shall be using portable PCs with high capacity or with wideband communication to a central system.

Keywords: Plant identification by computer, identification keys, multi-access keys, computerised floras

INTRODUCTION

A key for identifying a plant has traditionally meant a step-by-step elimination question and answer procedure, involving the characters of some group of plants, and set out on paper. It is also known as a diagnostic key, or when its questions are in pairs, a dichotomous key, and the first unambiguous examples seem to be the 'analytical tables' in the Flora of France by Lamarck (1778). Although hand-written keys have been used in the hand for more than two centuries, the future of identification surely lies in the use of computers.

CLASSICAL IDENTIFICATION

Identification is a routine and important activity in field botany, and it needs to be both quick and reliable. There are three approaches to this problem, and in order of practicality, these are

1 to know what the plant is already
2 to ask somebody else who already knows what it is. This point was illustrated by showing a photograph of *Senecio vulgaris,* which the President identified immediately, and failing that
3 to use a key
4 to match with a picture or specimen

Printed diagnostic keys are certainly quick, but are prone to error unless the user is thoroughly well acquainted with the characters. Where the British vascular flora is concerned, we have numerous keys available, although most of them require us to have flowering and/or fruiting material. Keys are not the only method for identification, but not surprisingly the more reliable methods tend to require a greater effort on the part of the user. Probably the most effective method is the multi-access key, or polyclave, as described below.

COMPUTER PROGRAMS FOR IDENTIFICATION

The first computer program for identification was published by Boughey *et al.* (1968) and the first key-constructing program was written by Pankhurst (1970). The latter was later expanded into the program package called PANKEY. This name was initially a joke, but can also be explained in terms of 'pan' meaning 'universal' and 'key' for 'identification'. All these programs are based on a standard format for coding descriptions called DELTA (short for Description Language for Taxonomy) invented by Dallwitz (1980). Table 1 lists these programs and their functions (Pankhurst, 1991).

TABLE 1. THE PANKEY PROGRAM PACKAGE

- DEDIT, a special purpose editor for descriptive (DELTA) data
- Construction of diagnostic keys, both automatically and interactively
- Construction and printing of taxon descriptions
- Expert interactive identification, with colour images
- Identification by comparison (or matching)
- Character analysis, for correlation and information content
- Conversion from DELTA to other data formats, for clustering or cladistics

An important motive for the development of this software was the difficulty, when expert help is not on hand, of identifying plants in the so-called 'critical' genera, where there are large numbers of similar taxa. This led to the development of DELTA data sets for a variety of genera in the British flora, such as Rubus (1973), Taraxacum (1976 & 1995 in Table 2), Euphrasia (1977), vegetative grasses (Pankhurst & Allinson, 1985) and Carex (Pankhurst & Chater 1988). Illustrations of the taxa and their characters greatly enhanced computerised keys as computer graphics and colour displays became available in the 1980's. An early example of this was the key to British orchids (Pankhurst 1989).

MULTI-ACCESS KEYS

These originally took the form of packs of punched cards, which were also known as 'polyclaves' and were first used for timber identification. The grass key (Pankhurst & Allinson 1985) is an example of the body-punched type of polyclave, and was actually computer generated, although this technology is now obsolete. The modern form of this is a computer program, also known as an interactive or online key. Although these programs still work by a process of sequential elimination of taxa by the addition of characters, they are quite different from diagnostic keys in other important ways (Table 2), and it is perhaps misleading to refer to them by the word 'key'.

TABLE 2. ADVANTAGES OF MULTI-ACCESS KEYS

1 Can use any character in any order
2 Different strategies for choosing characters
   a) Best = most informative
   b) Diagnostic = is it taxon X?
   c) Personal choice
4 Errors are tolerated
5 Measure of agreement
6 Characters can be changed at any time
7 Illustrations of characters and taxa, and online help

A multi-access key leaves the user free to use any character and in any order. This is in contrast to the diagnostic key, where you can only use the specified characters in the given fixed order. With all freedoms comes responsibility, so here the user will need to choose a strategy. The simplest procedure is to choose the characters subjectively, according to whatever seems easiest or most obvious. This is often a poor strategy because the chosen characters may not be very informative and as a result progress is often slow. If characters with high information content are chosen, then progress is usually more rapid. The BEST command in PANKEY selects those characters with the highest separation power i.e. those which separate the largest number of pairs of taxa, and displays them in descending order. The user is still free to choose whatever seems the most convenient character from this list, but progress is generally faster if one with a high score is chosen. As each character is described, the number of discriminating and unused characters falls, and the BEST list changes every time. If the user already has an idea of what the taxon the specimen might be then the third strategy is to use the DIAGNOSE command, where characters are requested for their ability to discriminate one chosen taxon from the rest. Whichever of these strategies is used, any character already described can be deleted again, so the key goes backwards just as easily as it goes forward. Lastly, a multi-access key permits the user to make errors, within reason. A limit can be set, which is the number of characters which must disagree before a taxon is eliminated. For example, if the limit is set to 2, then all taxa which disagree with the specimen as described so far by more than 2 characters will be rejected. This also means that up to 2 characters can be wrongly recorded without eliminating the correct answer, provided that the system contains sufficient alternative differentiating characters. If the user goes on increasing the agreement limit and adding further characters, then the value of the limit becomes a measure of confidence in the result, and the higher the better. Until all the available characters have been used the user can continue indefinitely and it is up to the user to decide when he/she is satisfied. Hence, unlike the printed diagnostic key, there is no fixed stopping point. Printed keys can also be provided with help and illustrations, but this can be done very easily in a computerised multi-access key.

There are websites which provide diagnostic keys, which is a good way of distributing them, but provides nothing more. Computer programs have been written to automate the functioning of diagnostic keys, but there seems little point in this, as all their disadvantages are preserved. Since the first multi-access identification program (Boughey *et al.* 1968) there has been a great deal of program development, and without wanting to review them all, there are or have been several hundred such programs, designed either for use on a desktop or, more recently, on web pages. Some of these programs are frankly re-inventive or derivative, and time would have been better spent on creating data sets instead. With the rise

of Microsoft Windows and other similar operating systems more attention has been given to the design of the user interface, but it is suggested that the basic functionality of the program, i.e. what it does and how well it does it, is what is really important. The example shown here (Fig. 1) is from PANKEY and is described in detail in Pankhurst (1991).

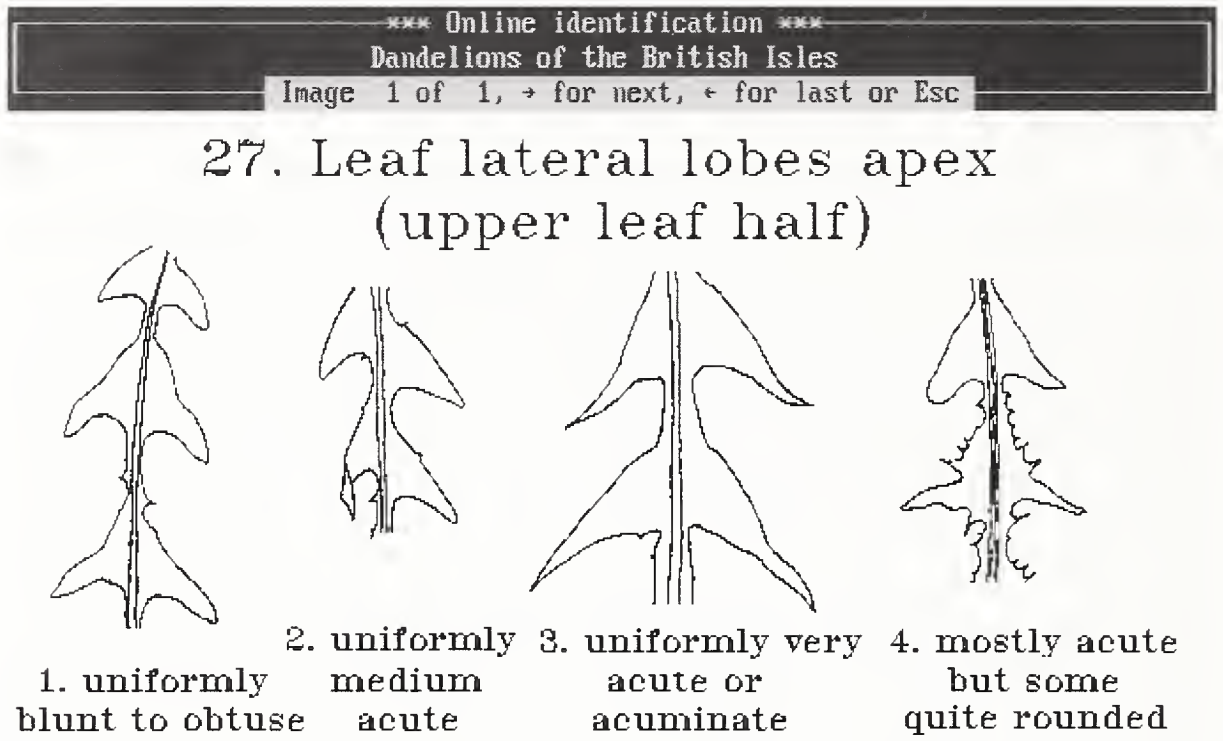


FIGURE 1. An illustrated character for interactive identification of *Taraxacum* (Key to British Taraxaca by R.J. Pankhurst (unpublished). A screen from the PANKEY Online identification program, illustrating *Taraxacum* leaves.

Other important examples are the INTKEY program, part of the DELTA system (Dallwitz 1980), and its competitor LUCID (Young *et al.* 1997). Several websites with identification programs have been created in recent times by BSBI members. Worthy of particular mention are the Science and Plants for Schools (SAPS) site 'Key for identifying British trees and shrubs' (see Fig. 2) due to the late Franklyn Perring and described at another talk during this conference, and the keys to *Euphrasia* and other groups by Quentin Groom (see Fig. 3). Outside the British Isles, the FloraBase project in Western Australia is attempting to provide a key to the whole flora of the area (see Fig. 4).

FLORAS ON CD-ROMS

There are an increasing number of CD-ROMs being produced for the floras of different areas, such as Europe, Finland, the Netherlands, Flora Iberica, Switzerland (see Fig. 5) and Poland, and most recently, the DVD-ROM created by ETI in Amsterdam for Clive's *New Flora of the British Isles* (Stace 1997) and described elsewhere in this book. I have the honour of working on the computer key for Clive's Flora DVD-ROM, although its incorporation is going to be delayed until a later edition. A computerised Flora might be more or less identical to the book version, as it is for *Flora Europaea* (Jorna 2001). Another useful

How to use the key to identify an unknown leafy twig

To use the key, you have to answer three questions about your leafy twig.

FIRST

Are the leaves simple? or compound?

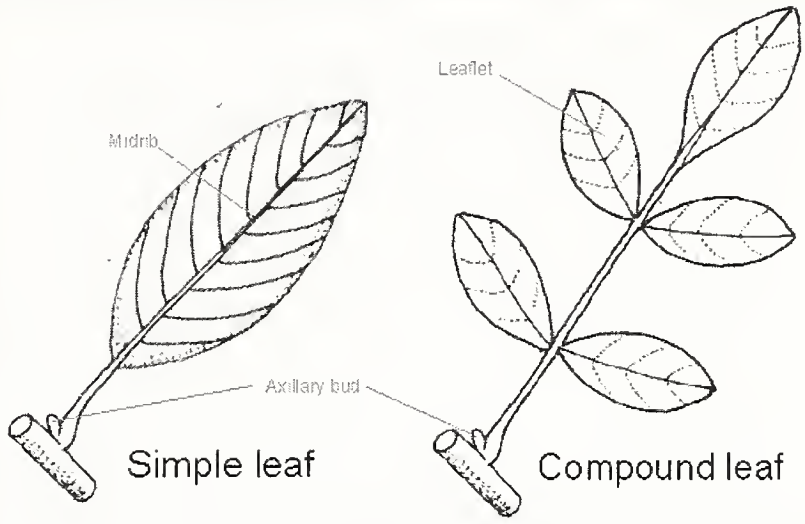


FIGURE 2. Identification of a British shrub (SAPS (Science and Plants for Schools) website www-saps.plantsci.cam.ac.uk/trees/home.html 'A Key for Identifying British Trees and Shrubs' by F. Perring *et al.*)

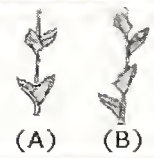
How long are the cauline leaves (mm)?

The leaves of *Euphrasia* can be divided into two types. The upper ones with a flower or capsule in the axils of the leaves and lower ones not associated with flowers. The cauline leaves are the lower ones without flowers. Measure the length (mm) of the larger leaves from their base to their tip.

Are the lower floral leaves alternate?

- I do not know
- Yes
- No

In general the leaves of Euphrasia are held on the stem in opposite pairs (A). However, the lower floral leaves can be alternate (B). If they are, answer yes to this question.



Are the leaves hairless?

- I do not know
- Yes
- No

To see the hairs on a *Euphrasia* leaf requires a hand lens. Look on both sides of the leaf for hairs, before deciding on your answer.

FIGURE 3. Identification of a *Euphrasia* (BSBI website - page from 'Identify a Plant' by Quentin Groom)

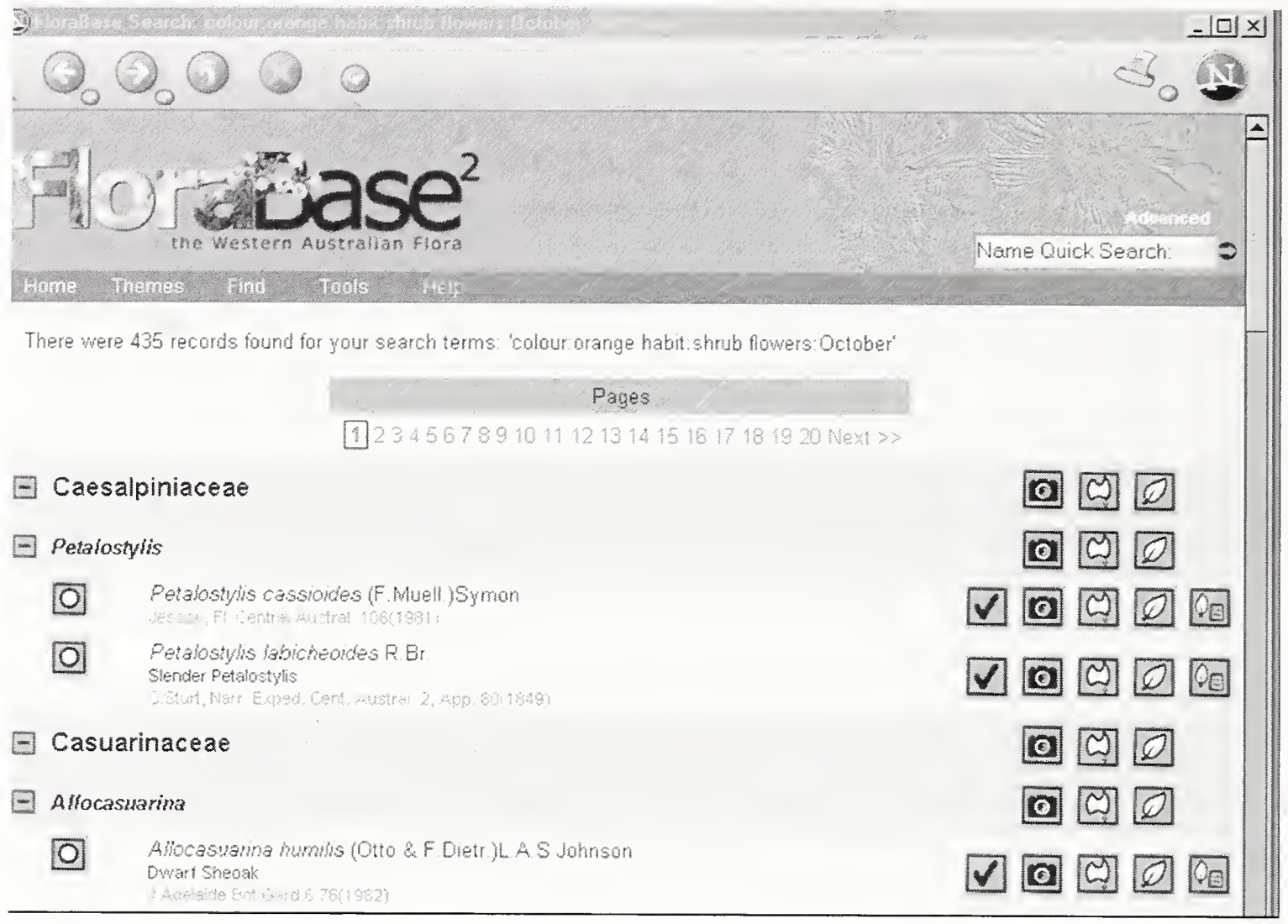


FIGURE 4. Flora of Western Australia; picture from www.florabase.calm.wa.gov.au

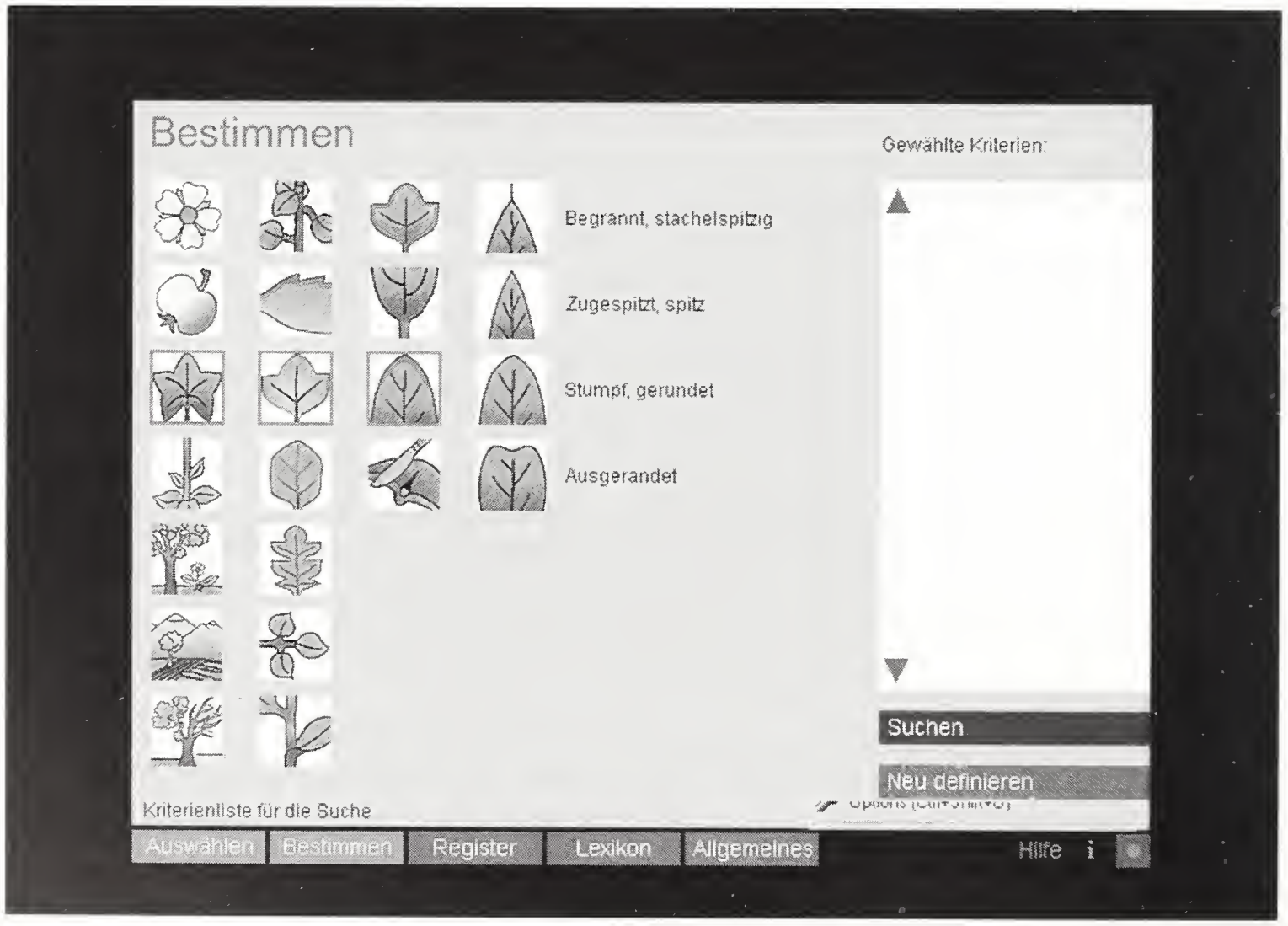


FIGURE 5. *Flora Helvetica* identification using leaf shape; picture from CD-ROM

approach would be to compile a set of named photographs of plants. Some of these projects have compiled a computer key to the flora, such as the Flora Helvetica (Lauber & Wagner 1996). A screen from this is shown in Figure 5. Although the presentation is very good, the key does not take you very far into the naming of any actual taxon because it does not contain enough characters to separate every taxon from every other, and you are left to search through the taxon images.

To provide enough characters and states to distinguish all taxa in the British flora would involve a large numbers of characters. For a selection of 50 taxa from the British flora, Pankhurst (1983) found 330 characters in the family key of edition 2 of *Flora of the British Isles* (Clapham *et al.* 1962). These 50 taxa were chosen one from each Order described in the Flora in order to have as much diversity as possible. Does this mean that the descriptions of each taxon are going to be impossibly large? Not so, in fact, because of the dependency between characters. In the 1983 project, each individual taxon was described adequately by a selection of somewhere between 20 and 50 characters out of the 330. Table 3 shows a hierarchy of dependent characters for hairs on stem leaflets. This happens because it is not unless the plant has an (aerial) stem and that stem leaves are present, and so on through a series of statements of the form 'if such an organ is present, then a certain character is possible', that hairs on the leaflets are a logical possibility. These dependency rules are expressed as data which is built into the DELTA format so that the algorithms can make considerable economies by knowing which characters are possible and which are inapplicable, and need not be scored.

TABLE 3. A HIERARCHY OF DEPENDENT CHARACTERS FOR 'LEAFLETS DENSELY HAIRY BELOW'

Aerial stem <presence>	= absent
	= present ✓
Stem leaves <presence>	= absent
	= present ✓
Stem leaves <compound>	= simple
	= compound ✓
Leaflets <hairy>	= glabrous
	= hairy ✓
Leaflets hairy <where>	= below ✓
	= above
	= both sides
Leaflets hairs below <density>	= sparse
	= dense ✓

Experience so far with the Stace Flora suggests about 1500 characters will be needed to separate the genera. The characters are being extracted from the family and generic keys and the text by using a specially written 'data mining' program which will be the subject of another paper. This data mining software is founded on a relational database of the flora and is already provided with all the scientific names and the hierarchy of their classification. It separates the characters into the structures e.g. stem, leaf, flower and the descriptors e.g. size, colour, roughness of which they are composed. So for example, 'petal colour' is made up of the structure 'petal' (which is part of the structure 'flower') and the descriptor 'colour' of type 'appearance'. This breakdown allows some standardisation of the characters, so that for example, the way that the hairs on the stem are described may draw from the same set of

states as are used for the hairs on the fruit. Such standardisation must not of course sacrifice any of the descriptive power of the original text, nor ignore the actual diversity of the plants themselves. This is partly achieved by associating a list of possible descriptive states with a descriptor, and then picking out a subset of these for use with each particular structure. The work being done here will create a character set specifically for Clive's Flora, and will only represent one possible way in which this might be done. In the future, other rival character sets for representing specific floras could emerge.

CONVERSION OF THE STACE FLORA

Clive's Flora is a concise Flora, which is to say that it deliberately sets out to provide the essential minimum of information, so that every plant that you might encounter in Britain can be recognised reliably with as little information as possible. To have done this is a considerable achievement. A second motivation for conciseness is to reduce the size of the resulting book (so that you can carry it in a rucksack), and therefore to reduce its cost. Once the Flora is moved onto a computer, however, the limitations of space related to cost immediately vanish. Permanent disc memory in modern desktop computers is now very spacious and cheap, and a blank DVD-ROM capable of holding 4 gigabytes costs about £2 sterling, regardless of what data it contains, and a CD-ROM of about 600 megabytes costs much less. There are still reasons why conciseness is a virtue and will be needed on occasion, but it should now be possible to include in the Flora all manner of useful data for which previously there was no room. Table 4 lists some of these possibilities.

TABLE 4. POSSIBLE CONTENTS OF A PORTABLE FLORA DATABASE

- National nomenclatural data (Kent list)
- Computerised descriptions of all taxa and all characters; vegetative, flowers, fruits, seeds and seedlings
- Ecological data
- Images; colour photos and drawings of plants and characters, and lots of them!
- Records; National (Atlas 2000)
  Census catalogue
  Individual records from VC databases
  VC distribution maps and old record cards
- Maps; OS plus relief, climate, geology, vegetation
- National Vegetation Classification (NVC) and identification of vegetation types
- Built-in GPS

In order to be able to select and retrieve such information, a stronger form of organisation is needed than simply the page-sequencing and indexing of a book. ETI are doing this by what is called 'hypertext' or the linking of sections of text with pointers to other sections of related text, which is a kind of text-oriented database. A stronger way of doing this is to use a properly organised relational database. There is no better known way of organising data so that you can navigate through it and retrieve relevant and related information, so one may expect computerised Floras to be organised in this way in the future.

HARDWARE

The conventional desktop computer already has enough memory to store enough data, not just text and numerical data but coloured images as well. How about taking this into the field? Richard Pryce has already been experimenting with pocket computers for botanical recording, but he tells me that their memories are too small at present to contain a complete Flora. The alternative is to carry a wireless-enabled terminal so that the data that you need can be received from a geo-stationary satellite. That is not really practical just yet either. But perhaps within 10 years or so, one way or another, a computerised flora will be practical in the field, and not just indoors. It will be tremendously exciting.

REFERENCES

BOUGHEY, A.S., BRIDGES, K.W. & IKEDA, A.G. (1968). An automated biological identification key. University of California, Irvine. Research Series no. 2.

CLAPHAM A.R., TUTIN T.G. & WARBURG E.F. (1962) *Flora of the British Isles*, edn 2. Cambridge University Press.

DALLWITZ, M.J. (1980). *User's guide to the DELTA System: a general system for encoding taxonomic descriptions*. CSIRO Division of Entomology, Canberra.

JORNA, S. (ed.) (2001). *Flora Europaea on CD-ROM* Cambridge University Press.

LAUBNER, K. & WAGNER, G. (1996). *Flora Helvetica* and CD-ROM. Paul Haupt, Bern.

LAMARCK, J.B.P. (1778). *Flore Françoise*, first edition, Paris, Imprimerie Royale.

PANKHURST, R.J. (1970). A computer program for the generation of diagnostic keys. *Computer Journal* **12**: 145–151.

PANKHURST, R.J. (1983). The construction of a floristic database. *Taxon* **32(2)**: 193–202.

PANKHURST, R.J. (1989). A computer program with colour graphics to identify orchids. *Orchid Review* **97**(1144): 53–55, 67.

PANKHURST, R.J. (1991). *Practical Taxonomic Computing*. Cambridge University Press.

PANKHURST, R.J. & ALLINSON, J.M. (1985). *British Grasses, a punched-card key to grasses in the vegetative state*. Field Studies Council & British Museum (Natural History) FSC Occasional Publication no. 10.

PANKHURST, R.J. & CHATER, A.O. (1988). *Sedges of the British Isles*. Computer Key No 1. Botanical Society of the British Isles.

STACE, C.A. (1997). *New Flora of the British Isles*, edn 2. Cambridge University Press.

YOUNG, T., YEATES, D. & THIELE, K. (1997). *LUCID player instruction manual,* University of Queensland, Australia.

Current taxonomic research on the British & European flora
Bailey, J. & Ellis, R.G. (eds) 15–25 (2006), BSBI. London.

Population based approaches in the study of *Pilosella* Hill (Asteraceae): A new view of its taxonomy?

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ABSTRACT

We present a structure of the *Pilosella* populations occurring in the Krkonoše Mts, northern Czech Republic. Each basic species, hybridogenous species and recent hybrid is documented by its frequency, cytotypes, breeding system, and chloroplast haplotypes. We compare this structure with the situation in another mountain range, the Šumava Mts in the south western Czech Republic. Both regions have the same structure of hybridising species, but the resulting population are different. We deduce that random phenomena in the past and the residual sexuality of apomictic species has influenced the present population composition. Our results are discussed in connection with existing approaches to the taxonomy of *Pilosella*.

Keywords: breeding systems, cytotypes, chloroplast haplotypes, population structure, taxonomy

INTRODUCTION

The genera *Hieracium* and *Pilosella* (often considered as subgenera of *Hieracium*) belong to one of the most complicated groups of temperate flora. Their complexity is closely connected with the number of recognized species, but reflects also the number of underlying mechanisms involved (for *Pilosella* see review by Krahulcová *et al.* 2000). In both genera, the pattern of morphological variation is clearly reticulate. Both genera differ by several morphological characters, but also in some evolutionary trends: one of the most important is that hybridisation is common in *Pilosella*, but recent hybridisation in *Hieracium* is extremely rare.

Studies of hybridisation between *Pilosella* species began in the 19th century; one of the well-known papers being that by Mendel (1869); this era ended with the monumental book by Nägeli & Peter (1885). In spite of this there is a lack of population studies similar to those which elucidated processes involved in many agamic complexes, as summarized in Richards *et al.* (1996). In *Pilosella*, some studies were only carried out recently by Russian authors (Kashin *et al.* 1999, 2000a, 2000b) and also outside their native distribution area in New Zealand (e.g. Chapman *et al.* 2003). The probable reasons for this absence of deep insight are difficulties with the determination of plants and the complexity of populations.

Recent methodological advances have allowed us to look at *Pilosella* populations using different approaches. In cooperation with German and other Czech colleagues we looked at *Pilosella* occurring in the Krkonoše Mts, in the western part of the Sudeten Mts; this area was already extensively studied in 19th Century and many hybridogenous species were described from it (results were summed by Nägeli & Peter 1885 and Schneider 1888-1895).

We tried to explore the existing variation at the levels of the population and the mountain range. We also started to compare this situation with another region outside the Sudeten Mts, the Šumava/Böhmerwald, a mountain range forming the border between Germany and the Czech Republic. Our approach included especially the determination of chromosome numbers and breeding systems (summarized by Krahulcová *et al.* 2001), hybridisation experiments (Krahulcová, unpublished data), residual sexuality of facultative apomicts (Krahulcová *et al.* 2004 and unpublished data), haplotype determination (Fehrer *et al.* 2005, Krahulec *et al.* 2004) and clonal structure detected by isozyme spectra and DNA fingerprinting (Fehrer *et al.* 2005, Krahulec unpublished data).

Knowledge of the detailed structure of the apomictic complexes within the two areas enabled us to search for common features. The aim of the present paper is to show these common features and to discuss the possibilities of some pragmatic, reasonable taxonomy of this complicated genus.

THE STUDY AREAS

The plants studied occur mainly in montane meadows in Central Europe. The montane meadows in the Krkonoše Mts were established in late medieval times. They are situated at altitudes between 600 and 1300 m, being relatively species rich, especially on a fine scale. The other region, the Šumava Mts, belongs to the Herzynian system. The habitats of the *Pilosella* species here are partly meadows and partly various successional stages with disturbed soil surfaces situated between 780 and 1150 m. Both mountain areas are isolated, and are separated across the Czech basin by more than 250 km (for location see Figure 1).

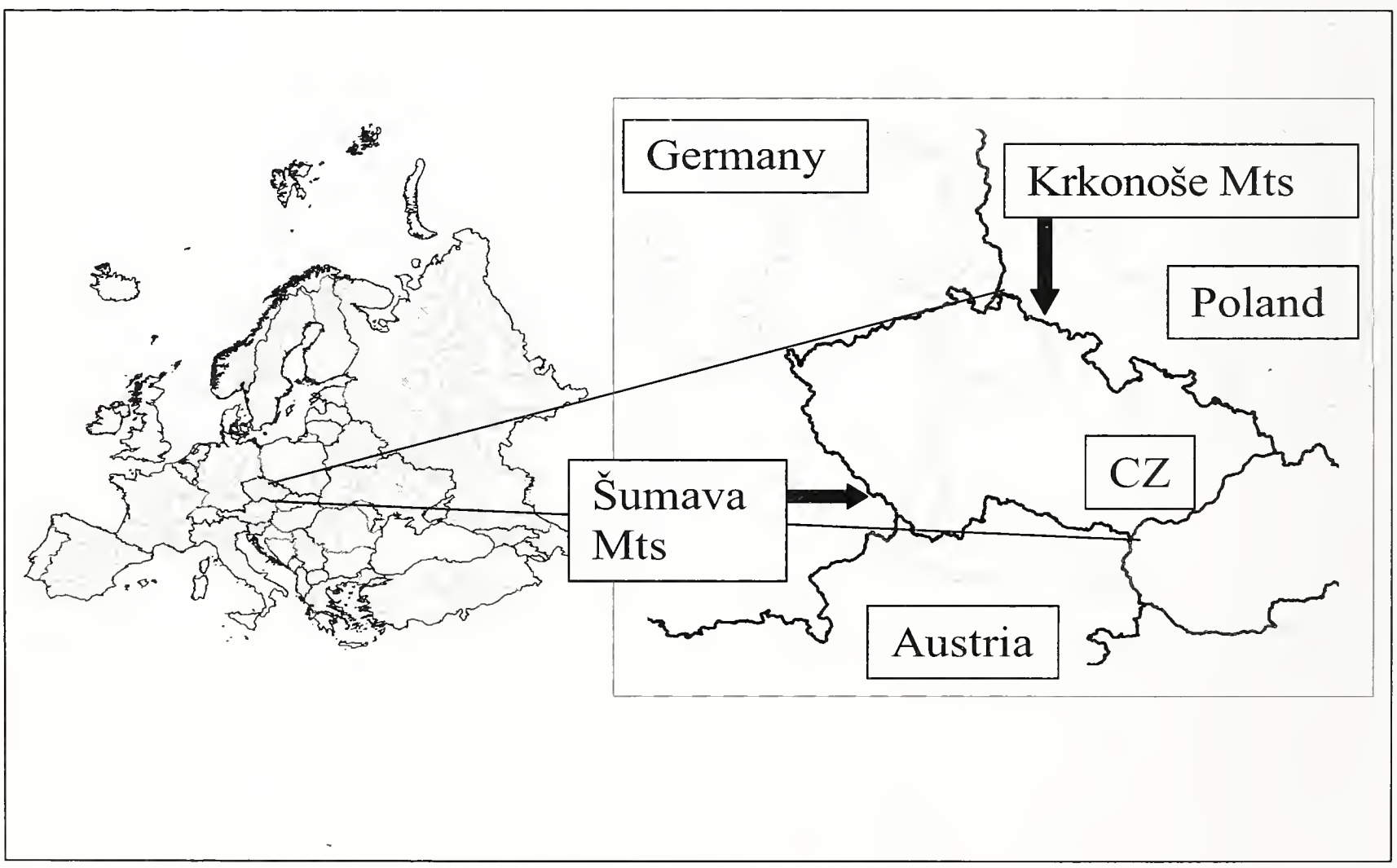


FIGURE 1. Location of the Krkonoše Mts and Šumava Mts, where the populations were studied

THE COMPLEXES STUDIED

The whole complex occurring in the Krkonoše Mts is rather complicated. For simplicity we divided it into two parts, the yellow-flowered and orange-flowered (red) groups. Both have *P. officinarum* (*H. pilosella*) as one parental species (a sexual one). This putative origin has been checked, especially by experimental hybridisation. Apomixis in *Pilosella* is of the aposporous type; it is facultative. When we say that the breeding system is apomictic, it is apomictic with some degree of residual sexuality.

A complete list of species discussed is in the Appendix (p. 25), together with their equivalents in *Hieracium.*

RESULTS

THE KRKONOŠE MOUNTAINS

The whole complex is formed from six basic species, their hybrids behaving as hybridogenous species, and secondary hybrids (Figs 2 & 3 (left)). Two of them are diploids (*P. auricula, P. onegensis*), the others are tetraploids to hexaploids, and we found one heptaploid plant. Some of the species are represented by one ploidy level, others by two ploidy levels and *P. piloselliflora* by three ploidy levels.

With respect to the breeding system, three species were found to be fully sexual: *P. auricula, P. officinarum, P. onegensis*. The tetraploid cytotypes of two other species, *P. piloselliflora* and *P. schultesii,* were recorded as both sexual and apomictic. All other species (and cytotypes at higher ploidy level) were apomictic (Krahulcová & Krahulec 1999, 2001 and unpublished results).

Two main haplotype groups were distinguished, each of them with two subtypes differing in shorter sections of cpDNA (Fehrer *et al.* 2005). The following basic species share individual haplotype groups, whose presence in individual hybrids is indicated in Figures 2 & 3 (left).

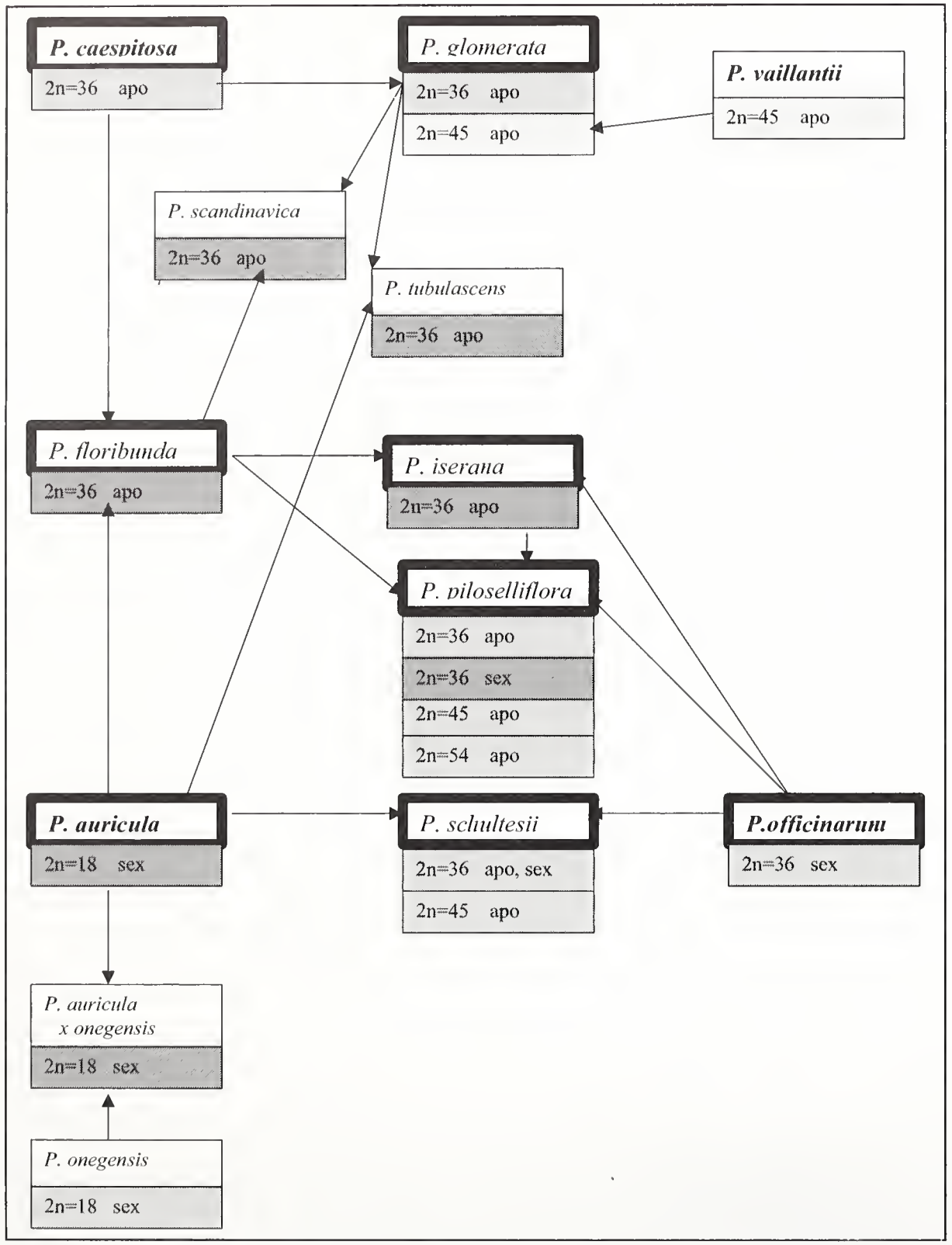
Haplotype group 1: *P. auricula, P. aurantiaca* (pentaploid). Subtype characterized by deletion of 5bp was found in tetraploid *P. aurantiaca.*

Haplotype group 2: *P. onegensis, P. caespitosa, P. officinarum*; subtype characterized by 6bp insert is present in *P. cymosa* (here represented by subsp. *cymigera*).

Besides the sexual *P. officinarum*, a key hybridogenous species *P. floribunda* is also involved in the formation of other hybrids (Fig. 2). It is tetraploid and always apomictic.

The hybrid morphologically closer to *P. floribunda* (*P. iserana*), mostly tetraploid and apomictic, is evidently an n+n hybrid with *P. floribunda* as the mother plant. Artificial hybridisation between *P. floribunda* and *P. officinarum* was successful only in one direction, using apomictic *P. floribunda* as a mother plant, which is also supported by the identical haplotype of *P. floribunda* and *P. iserana*. *P. iserana* has low morphological as well as molecular (DNA fingerprinting) variation.

The type morphologically closer to *P. officinarum* (*P. piloselliflora*) is variable in all respects, i.e. in morphology, ploidy levels and breeding systems. It is also variable in haplotypes, which indicates that both parents, *P. iserana* (or rarely *P. floribunda*) and *P. officinarum*) served as mother plants. Many isozyme phenotypes were found, even at the locality level. All these facts indicate that *P. piloselliflora* is a complex of genotypes repeatedly formed by hybridisation between *P. iserana* (or *P. floribunda*) and *P. officinarum*. The hybridisation between *P. iserana* and *P. officinarum* (in both directions) is easily achieved and was repeated several times during garden experiments.



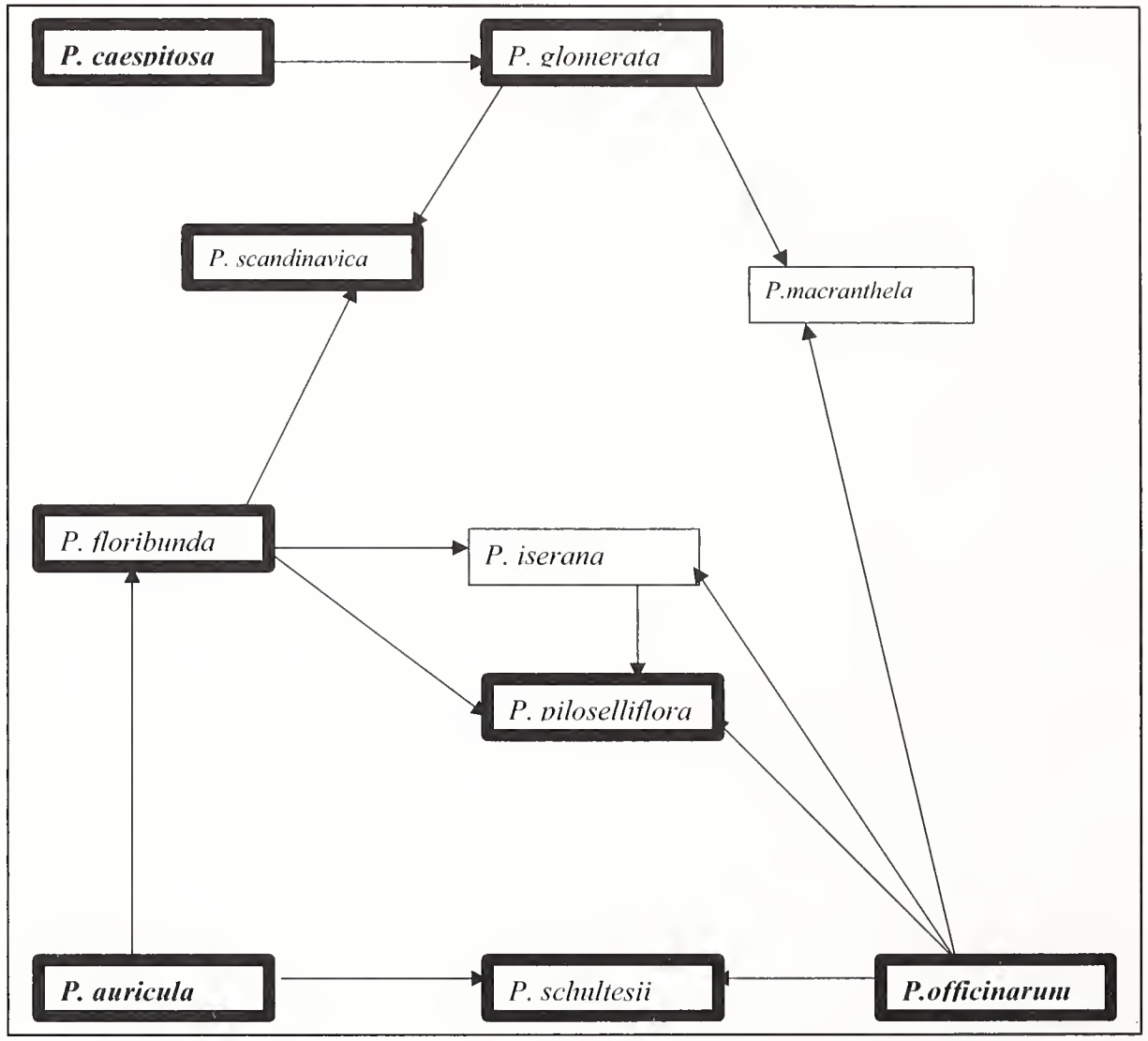


FIGURE 2. The strueture of the populations of the yellow part of the eomplex in the Krkonose Mts. (left) and in the Sumava Mts. (right). Frequent types being observed in more than two localities have a bold frame. Names of the basic speeies are given in bold; arrows going from their parents indieate origin of hybrids. For all speeies, hybridogenous species, and reeent hybrids their eytotypes, breeding system and ehloroplast haplotype (by different shading of box with ehromosome numbers) are given.

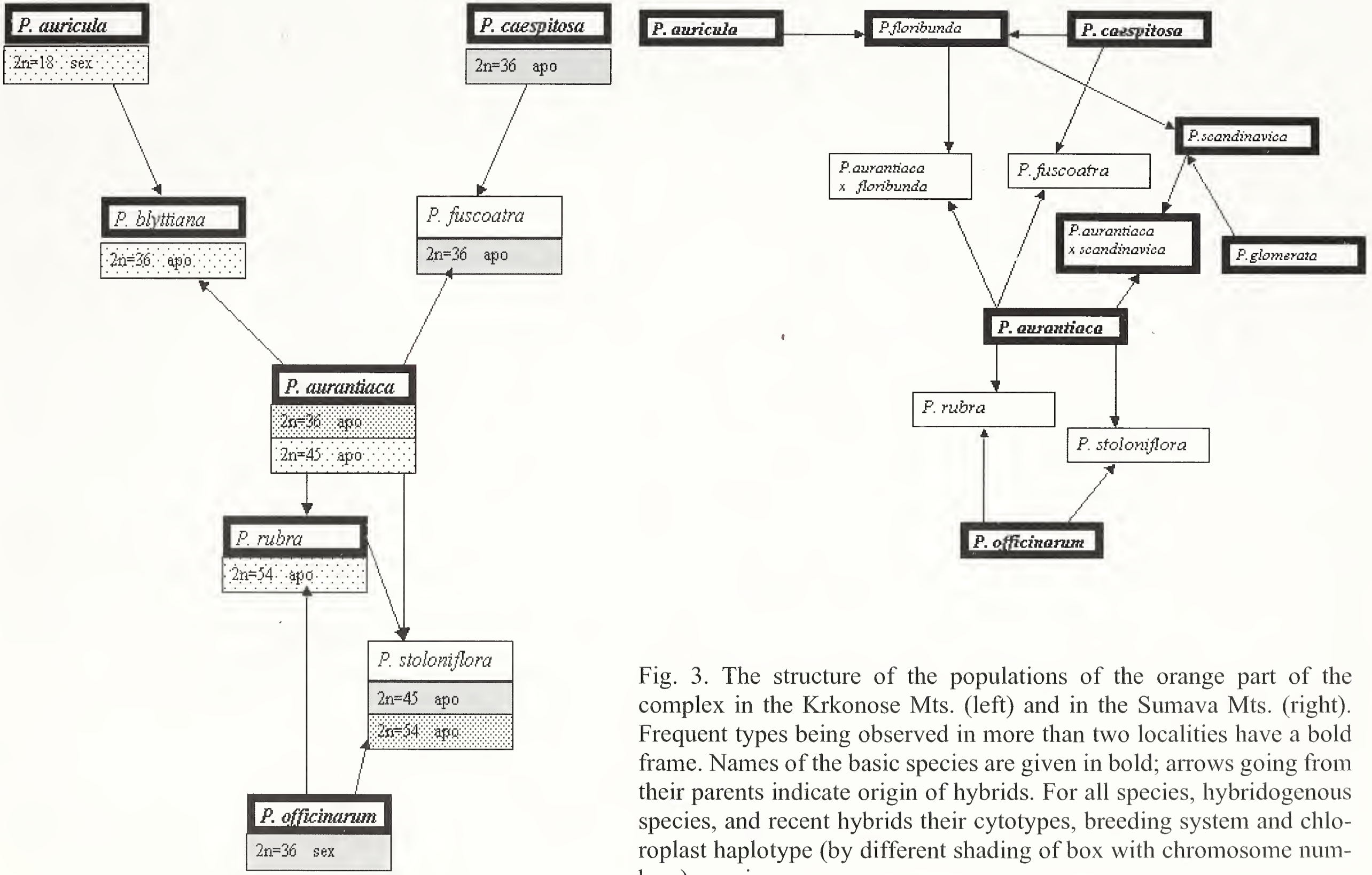


Fig. 3. The structure of the populations of the orange part of the complex in the Krkonose Mts. (left) and in the Sumava Mts. (right). Frequent types being observed in more than two localities have a bold frame. Names of the basic species are given in bold; arrows going from their parents indicate origin of hybrids. For all species, hybridogenous species, and recent hybrids their cytotypes, breeding system and chloroplast haplotype (by different shading of box with chromosome numbers) are given.

Triploid hybrids between *P. officinarum* and *P. auricula* were not found in the field; we found tetraploid and pentaploid plants, the first being sexual or apomictic. These are similar to *P. piloselliflora*.

The orange-flowered complex consists of hybrids between *P. aurantiaca* and the yellow-flowered species, *P. caespitosa*, *P. auricula* and especially *P. officinarum* (Fig. 3 left). Within the region, *P. aurantiaca* occurs as a common tetraploid (two isozyme phenotypes) and a rarely as a pentaploid, both apomicts. The frequency of its hybrids is variable: its hybrid with *P. caespitosa* (*P. fuscoatra*) was found for the first time at the end of 1970s and has a limited distribution. Its hybrid with *P. auricula* (*P. blyttiana*) has been known from the area for more than 100 years (and it was independently described as *H. latibracteum* Peter). It is rare at present, without any morphological or DNA (fingerprinting profiles) variation; evidently, there is only one clone present.

The variation of hybrids between *P. aurantiaca* and *P. officinarum* is more complicated. One type closer to *P. aurantiaca* was described from this region as *H. rubrum* (*P. rubra*). It is apomictic, morphologically as well as cytologically uniform (hexaploid). Its cp-haplotype differs from that of the common tetraploid *P. aurantiaca* forms, but is the same as that of the pentaploid *P. aurantiaca*. A direct relationship with present tetraploid *P. aurantiaca* seems improbable. We have so far detected two clones by DNA fingerprinting and isozyme phenotypes.

The types closer to *P. officinarum* (*P. stoloniflora*) are more variable, both cytologically (pentaploids- and hexaploids) and morphologically. One pentaploid plant was found within the stand of *P. rubra*. It probably originated as a product of back-cross between *P. rubra* and *P. officinarum*, morphologically corresponding to hybrids of experimental origin. Hexaploids have the same cp-haplotype as *P. rubra* and pentaploid *P. aurantiaca*. So, in spite of our efforts the exact origin of these types remains unclear, as there are several possible routes.

SUMMARY OF THE STRUCTURE OF THE COMPLEX IN THE KRKONOŠE MTS

1 A number of hybrids where predominantly an apomictic plant was the mother. They were either formed by hybridisation between two apomictic types, or an apomictic type was the mother in those hybrids coming from the crosses between apomictic and sexual plants. This indicates that residual sexuality is an important factor shaping the structure of an agamic complex.
2 In both the yellow and orange-flowered parts of the complex, there are features common to the hybrids between sexual *P. officinarum* and apomictic species (*P. floribunda, P. aurantiaca*), forming morphologically different types. Hybrids closer to the apomictic parent are morphologically and karyologically uniform, while others closer to the sexual parent are more variable with respect to morphology, cytotypes, clonal structure and, in the case of *P. piloselliflora,* also in breeding system.
3 Hybrids which are morphologically closer to the apomictic parent have always had apomictic type as mother. Hybrids closer to the sexual parent (*P. officinarum*) are variable with respect to cp-haplotype; it indicates that either parent served as a mother plant.

COMPARISON WITH THE ŠUMAVA MOUNTAINS.

The set of basic species involved is in general the same as in the Krkonoše Mts; *P. onegensis* and *P. vaillantii* are absent, but both are so rare that they do not influence the shape of the complex. We present here only data about presence and frequency of hybrids (Figs 2 & 3 (right)). In spite of the fact that the basic species are the same, there are different hybrids occurring with different frequency. Some of them originated recently (we found only individual plants of *P. rubra* and *P. stoloniflora*), and some others are common (*P. scandinavica*). Some hybrids are present in only one mountain range. It seems that the hybridisation of some species in the Šumava Mts is rare, as for example that between *P. floribunda* and *P. officinarum*. The rise of the successful (with respect to its abundance and widespread distribution) *P. iserana* in the Krkonoše area led to its subsequent hybridisation with *P. officinarum* and formation of many cytotypes and genotypes of *P. piloselliflora*. In spite of the common occurrence of *P. aurantiaca* and *P. auricula* there is nothing resembling *P. blyttiana* in the Šumava Mts. On the other hand, *P. aurantiaca* hybridises here with *P. floribunda* and *P. scandinavica;* these hybrids are absent in the Krkonoše Mts.

The comparison of the two mountains is summarized in the following points:

1 The same set of basic species forms a different pattern of hybrids in particular regions.
2 The different age of hybrids is reflected in their different distribution and frequency.
3 The same hybridogenous type (with regard to morphology, ploidy level, breeding system and genome constitution) can be an established and widespread in one region and a very rare recent hybrid in another region (e.g. *P. rubra* in the Krkonoše Mts and *P. scandinavica* in the Šumava Mts behave as established hybridogenous species).
4 Evidently, some rare hybridisation events in the past shaped the whole hybridogenous complex.
5 Some hybrids are usually unique, confined to individual localities. They represent with high probability recent hybrids.

CONCLUSIONS FOR TAXONOMIC TREATMENT AND DETERMINATION OF PLANTS

In our opinion the total population of the genus growing together in one geographic region should be considered as a whole. Firstly, the basic species should be recognized. The next step should be the recognition of the common plants of hybridogenous origin, which may shape the formation of other (secondary) hybrids. The determination of all plants collected in the field is often impossible without special analysis of their genetic markers, because progeny of some hybridogenous types is extremely variable and in different regions different types survive.

The complex pattern within *Pilosella* has attracted many taxonomists in the past. In our opinion our approach might help to support some of the existing philosophies. There are at least four different approaches that are still alive in Europe (Schuhwerk 2002):

One originated on the European continent and was summarized by the monumental book by Nägeli & Peter (1885), followed and further developed by Zahn (1922-1930); it is in general followed in most national floras in Central Europe: e.g. by Nyárády (1965), Gottschlich (1998), Bräutigam & Schuhwerk (2002), Schuhwerk & Fischer (2003), Chrtek in Kubát *et al.* (2002), Mirek *et al.* (2002). These authors distinguished basic species and intermediate ones, the latter being labelled by a formula indicating the origin and the quantitative influence of its particular parents. In addition, there is a rich structure of

infraspecific taxa, treated as grex (group of subspecies), subspecies, and variety. Recently, some authors have stressed (e.g. Schuhwerk 2002) that some of the hybrids are old, fully established types behaving as independent species with their own distribution area, but others are recent hybrids.

The second approach was developed in Scandinavia (Sweden, Finland) and Russia and is still used by Russian authors (e.g. Schlyakov 1989). They described each distinguishable entity as microspecies and paid little attention to possible relationships between these microspecies.

The third approach, developed by British authors Sell and West was used e.g. in *Flora Europaea* (Sell & West 1976). They considered as species the main species of continental botanists (with three exceptions, they included also *H. flagellare, H. sphaerocephalum* and *H. vahlianum* usually considered as hybrids in Central Europe). All other types were evaluated as hybrids, those having the same parental combination being synonymized. This approach fully corresponds to the ICBN. The resulting structure is simple and understandable, even for non-specialists. The main difficulty of this approach is the fact that in some regions only hybridogenous types occur.

Tyler (2001) has recently developed the fourth approach. It is based on the knowledge of Scandinavian types and on the fact of common hybridisation within *Pilosella* species. He formulated several rules (cf. Tyler 2001: 67, Schuhwerk 2002) to define levels of the species, subspecies and variety. Based on them he created systems involving both main and hybridogenous species under one species name. This approach substantially reduced the number of species, especially for Scandinavia, but complexity is still reflected at infraspecific level.

Our results reject the possibility that all distinguishable types can be classified. The number is too high to produce any reasonable system. They are repeatedly formed, because even in apomicts the degree of residual sexuality is not negligible.

The approach by Tyler (2001) reflects this high degree of hybridisation; however, our main objection to his system of subspecies is based on the facts demonstrated above. He overemphasized his Scandinavian experience and lumped together those species which often hybridise in Scandinavia. But if we use his approach consistently, it would be necessary to develop different and incompatible systems for different regions. The frequency of hybridisation is evidently influenced by the degree of sexuality (including the presence of fully sexual types), compatibility of co-occurring cytotypes, availability of suitable habitats, and some random events in the past. For those reasons we consider the Tyler's approach as not suitable.

The differences of the last two approaches are not so big as might appear. Both approaches use the corresponding categories: the basic species of Zahn and his successors correspond to species used by Sell and West. The main difference is in the treatment of intermediates of hybrid origin. Our experience showed that at least in some complexes it is possible to distinguish two types of hybrids, each closer to one of the parents. Further splitting seems impossible, because of high number of coexisting types, either within one region or in different regions. In fact, this approach has recently been accepted by Bräutigam & Schuhwerk (2002). Within the yellow-flowered group, whose structure we have shown, they included *P. apatelia* (*H. apatelium*) within *P. piloselliflora*. This is recommended because of continuous variation between *H. apatelium* and *H. piloselliflorum*, due to the high number of genotypes and variable breeding systems producing new types. The same seems to hold for the hybrids between *P. aurantiaca* and *P. officinarum*: *P. rubra* has low variation and also in other regions it is present as a hexaploid type (Schuhwerk & Lippert 1997, our

unpublished data from the Šumava Mts). On the other hand, *P. stoloniflora* represents a set of different hybrids with a higher influence from *P. officinarum* (both primary hybrids and backcrosses of *P. rubra*). We realize that this approach is not fully consistent with ICBN, because it is difficult to decide whether a particular type is a hybridogenous species or a hybrid. The main difficulty of this approach is that in other regions the hybridising species can differ in their ploidy level and other features and so the result of the same hybridisation can seem different. As an example we can use the occurrence of a hybrid similar to *P. iserana* in the Šumava Mts; this plant is a hexaploid and evidently has a different genomic constitution in comparison with the tetraploid *P. iserana* from the Sudeten. Evidently, the use of this approach would lead again to the formation of geographically limited systems, but these systems could be compatible provided they use the same basic species as a firm framework. Such systems are useful in some regions, as for Central Europe. Schuhwerk & Fischer (2002-2003) recently produced an excellent account for Austria.

Our experience from experimental hybridisation supports the Sell & West approach: hybrids differing in the proportion of parental genomes originate even from one cross, combining reduced and unreduced gametes (Krahulcová & Krahulec 2000, Krahulcová *et al.* 2004). Consequently, both approaches may be combined provided that they use the same species as a stable framework.

Evidently, we need more data about the structure of individual hybridogenous complexes from different regions. The present situation, when only limited number of regions (and species complexes) was studied in detail, does not allow us to make any final generalization. However, we think that it is necessary to define the basic species and their distribution areas. Even this process does not seem to have reached finality: e.g. diploid *P. onegensis*, which has an extensive distribution area from Central and southeastern Europe to western Siberia, is usually considered as a subspecies of *P. caespitosa*. Similarly, there are no fully clear relationships between *P. officinarum* and its diploid relatives such as *P. hoppeana*, *P. macrantha*, *P. peleteriana* and especially diploids occurring in southern Europe. Even the relationships between these diploids are unclear: e.g. *P. macrantha* is sometimes evaluated as a species, and sometimes as a subspecies of *P. hoppeana* (subsp. *testimonialis*). This definition of a set of basic species can form a firm framework for the development of a consistent system of hybrids, which can be used by non-specialists.

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REFERENCES

BRÄUTIGAM, S. & SCHUHWERK, F. (2002). *Hieracium* L. – Habichtskraut. In: E.J. JÄGER & K. WERNER (eds), *Exkursionsflora von Deutschland. Gefässpflanzen: Kritischer Band*, p. 709–734.

CHAPMAN, H., HOULISTON, G.J., ROBSON, B. & ILINE I. (2003). A case of reversal: The evolution and maintenance of sexuals from parthenogenetic clones in *Hieracium pilosella*. *Int. J. Plant. Sci.* **164**: 719–728.

CHRTEK, J. JUN. (2002). *Hieracium*. In: K. KUBÁT *et al.* (eds), *Klíč ke květeně České republiky*, Academia, Praha, pp. 206–232.

FEHRER, J., ŠIMEK, R., KRAHULCOVÁ, A., KRAHULEC, F., CHRTEK. J., BRÄUTIGAM, E. & BRÄUTIGAM, S. (2005). Evolution, hybridization, and clonal distribution of apo- and amphimictic species of *Hieracium* subgen. *Pilosella* (Asteraceae: Lactuceae) in a Central European mountain range. In: F.T. BAKKER, L.W. CHATROU, B. GRAVENDEEL & P.B. PELSER: Plant species-level systematics: patterns, processes and new applications. *Regnum Vegetabile* **143**: 175-201. Koeltz, Königstein.

GOTTSCHLICH, G. (1998). *Hieracium*. In: R. WISSKIRCHEN & H. HAEUPLER, *Standartliste der Farn und Blütenpflanzen Deutschlands*, pp. 245–263.

KASHIN, A.S. *et al.* (1999). Speciation potential of the *Pilosella* (Asteraceae) agamic complex. 1. Base species. *Bot. Zhurn.* **84(4)**: 25–38.

KASHIN, A.S., ZALESNAYA, S.V., TITOVETS, V.V. & KIREEV, E.A. (2000). Speciation potential of the *Pilosella* (Asteraceae) agamic complex. 2. Natural interspecies hybridisation. *Bot. Zhurn.* **85(3)**: 1–13.

KASHIN, A. S., ZALESNAYA, S. V. & TITOVETS, V.V. (2000). Speciation potential of the *Pilosella* (Asteraceae) agamic complex. 3. Genomic variability in populations and in separate plant progenies. *Bot. Zhurn.* **85(12)**: 13–28.

KRAHULCOVÁ, A. & KRAHULEC, F. (1999). Chromosome numbers and reproductive systems in selected representatives of *Hieracium* subgen. *Pilosella* in the Krkonoše Mts (the Sudeten Mts). *Preslia*, **71**: 217–234.

KRAHULCOVÁ, A. & KRAHULEC, F. (2000). Offspring diversity in *Hieracium* subgen. *Pilosella* (*Asteraceae*): New cytotypes from hybridisation experiments. *Fragm. Flor. Geobot* **45**: 239–255.

KRAHULCOVÁ, A., KRAHULEC, F. & CHAPMAN, H.M. (2000). Variation in *Hieracium* subgen. *Pilosella* (*Asteraceae*): what do we know about its sources? *Folia Geobot.* **35**: 319–338.

KRAHULCOVÁ, A., KRAHULEC, F. & CHRTEK, J. (2001). Chromosome numbers and reproductive systems in selected representatives of *Hieracium* subgen. *Pilosella* in the Krkonoše Mts (the Sudeten Mts) – 2. *Preslia* **73**: 193–211.

KRAHULCOVÁ, A., PAPOUŠKOVÁ, S. & KRAHULEC, F. (2004). Reproduction mode in the allopolyploid facultatively apomictic hawkweed *Hieracium rubrum* (Asteraceae, *H.* subgen. *Pilosella*). *Hereditas* **141**: 1–12.

KRAHULEC, F., KRAHULCOVÁ A., FEHRER J., BRÄUTIGAM S., PLAČKOVÁ I. & CHRTEK J. JUN. (2004). The sudetic group of *Hieracium* subgen. *Pilosella* in the Krkonoše Mts: a synthetic view, *Preslia* **76**: 223–243

MENDEL, G. (1869). Ueber einige aus künstliche Befruchtung gewonnenen *Hieracium*-Bastarde. *Verh. Naturf. Vereins Brünn* **8**: 26–31.

MIREK, Z., MIRKOWA-PIĘKOTŚ, H., ZAJĄC, A. & ZAJĄC, M. (eds), (2002). *Flowering plants and pteridophytes of Poland. A checklist*, Kraków.

NÄGELI, C. VON & PETER, A. (1885). *Die Hieracien Mittel-Europas. Monographische Bearbeitung der Piloselloiden mit besonderer Berücksichtigung der mitteleuropäischen Sippen.* München.

NYÁRÁDY, E.I. (ed.) (1965). *Flora Republicii populare Romîne X.* Bucureşti.

RICHARDS, A.J., KIRSCHNER, J., ŠTĚPÁNEK, J. & MARHOLD, K., (eds) (1996). *Apomixis and taxonomy*. Opulus Press, Uppsala.

SCHNEIDER, G. (1888-1895): Die Hieracien der Westsudeten. *Das Riesengebirge in Wort u. Bild*, Trautenau, Marschendorf, **8**: 75–80, 110–114 (1888), **9**: 18-24, 54–59, 83–100, 147–152 (1889), **10**: 21–31, 69–71 (1890), **11(1-2)**: 30–35 (1891), **11(3-4)**: 21–28 (1891), **12**: 23–25, 65–68 (1892), **13(1-2)**: 20–23 (1893), **13(3-4)**: 20–29 (1893), **14**: 21–28, 65–69 (1894), **15**: 17–21 (1895).

SCHLYAKOV, R.N. (1989). Rod 33. Yastrabinočka – *Pilosella* Hill. In: N.N. TSVELEV (ed.), *Flora Evropeiskoi časti SSSR* 8, Nauka, Leningrad, pp. 300–377.

SCHUHWERK, F. (2002). Some thoughts on the taxonomy of *Hieracium*. *Ber. Bayer. Bot. Ges.* **72**: 193–198.

SCHUHWERK, F. & FISCHER, M.A. (2003). Bestimmungsschlüssel der Untergattung *Hieracium* subg. *Pilosella* in Österreich und Südtirol. *Neilreichia* **2-3**: 13–58.

SCHUHWERK, F. & LIPPERT, W. (1997). Chromosomenzahlen von *Hieracium* (*Compositae, Lactuceae*) Teil 1. *Sendtnera* **4**: 181–206.

SELL, P.D. & WEST, C. (1976). *Hieracium*. In: T.G. TUTIN *et al.* (eds), *Flora Europaea* 4, Cambridge University Press, Cambridge, London, etc., pp. 358–410.

TYLER, T. (2001). The genus *Pilosella* in the Nordic countries. *Svensk. Bot. Tidskr.* **95**: 39–67.

ZAHN, K.H. (1922-1930). *Hieracium*. In: P. ASCHERSON & P. GRAEBNER (eds), *Synopsis der mitteleuropäischen Flora* **12(1)**, Leipzig.

APPENDIX

The *Pilosella* species studied with their equivalents in *Hieracium*

P. aurantiaca (L.) F.W. & C.H. Schultz	*H. aurantiacum* L.
P. aurantiaca × *P. floribunda*	*H. aurantiacum* × *H. floribundum*
P. auricula (L.) F.W. & C.H. Schultz	*H. lactucella* Wallr.
P. blyttiana (Fries) F.W. & C.H. Schultz	*H. blyttianum* Fries
P. caespitosa (Dumort.) Sell & West	*H. caespitosum* Dumort
P. floribunda (Wimmer & Grabowski) Arv.-Touv.	*H. floribundum* Wimmer & Grabowski
P. fuscoatra (Nägeli & Peter) Soják	*H. fuscoatrum* Nägeli & Peter
P. glomerata (Froel. in DC) Arv.-Tour.	*H. glomeratum* Froel. in DC.
P. macranthela (Nägeli & Peter) Soják	*H. macranthelum* Nägeli & Peter
P. officinarum F.W. & C.H. Schultz	*H. pilosella* L.
P. onegensis (Norrl.) Norrl.	*H. onegense* Norrl.
P. iserana (Uechtr.) Soják	*H. iseranum* Uechtr.
P. piloselliflora (Nägeli & Peter) Soják	*H. piloselliflorum* Nägeli & Peter
P. rubra (Peter) Soják	*H. rubrum* Peter
P. scandinavica (Dahlst.) R.N. Schlyakov	*H. scandinavicum* Dahlst.
P. schultesii (F. Schultz) F.W. & C.H. Schultz	*H. schultesii* F. Schultz
P. stoloniflora (Waldst. & Kit.) F.W. & C.H. Schultz	*H. stoloniflorum* Waldst. & Kit.
P. tubulascens Norrl.	*H. tubulascens* Norrl.
P. vaillantii (Tausch) Soják	*H. cymosum* L. subsp. *cymigerum* (Reichenb.) Peter

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Taxonomic complexity, conservation and recurrent origins of self-pollination in *Epipactis* (Orchidaceae)

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ABSTRACT

The recent post-glacial colonisation of Britain has given little time for the evolution of endemic plant species. The few British endemic species that have been recognised tend to occur in taxonomically complex groups that possess mechanisms promoting rapid diversification. Such taxonomic complexity leads to problems for plant conservation because a species has to be circumscribed and recognised before its distribution, status and threats can be established. One classic example of the challenges for conservation in taxonomically complex groups is the British endemic orchid *Epipactis youngiana*. This species is afforded full legal conservation protection but is one of a large number of taxonomically difficult species recently recognised in the genus; it is difficult to distinguish from the more common *E. helleborine,* and its species status has been questioned. We have used a combination of genetic markers (allozymes, chloroplast microsatellites and RFLPs) from a large sample set to establish the taxonomic and conservation status of *E. youngiana* and to place it in the wider context of patterns of breeding system variation and taxon differentiation in the genus. Our data provide evidence that recurrent breeding system transitions between cross-pollination and self-pollination are an important mechanism for diversification in the genus, and there are numerous genetically different taxa that are homozygous and uniform for different subsets of allelic diversity found in allogamous species. *E. youngiana* is the one major exception to this pattern. It has a floral morphology consistent with self-pollination, but has not achieved reproductive isolation from *E. helleborine*. The potential mechanisms underlying the recurrent evolution of self-pollination in *Epipactis* are discussed, as is the need for developing conservation strategies that reflect dynamic diversification in the genus, rather than the current heavily typological (is it distinct or not?) species-based approach.

Keywords: allozymes, cpSSRs, endemic, *Epipactis*, genetic markers, RFLPs, self-pollination, taxonomic complexity.

INTRODUCTION

The British flora has benefited from a long history of floristic study (Clapham *et al.* 1989; Stace 1991, 1997), two major mapping exercises based on over 9 million records (Preston *et al.* 2002), various accounts of species of conservation importance (Stewart *et al.* 1994; Wigginton 1999), detailed biological floras of individual species, a comprehensive evaluation of chromosome number variation, a vice comital flora (Stace *et al.* 2003), and an overview of hybridisation in the flora (Stace 1975). This enormous resource base provides an excellent foundation for taxonomic, ecological, evolutionary and conservation research.

Building on this resource base, the delimitation and identification of many species in the British flora is now considered routine. The recognition and description of clear-cut morphological differences between species has effectively led to a consensus on the most appropriate taxonomic treatment for a large number of genera. There are, however, some persistently troublesome groups of plants that defy attempts to achieve a widely accepted taxonomic treatment. These taxonomically complex groups contain a large proportion of Britain's endemic higher plant species (e.g. *Sorbus*, *Epipactis*, *Euphrasia*, *Ulmus*, *Hieracium*, *Taraxacum*, *Rubus*, *Limonium*).

Taxonomic complexities which affect the most appropriate delimitation of species cause problems for conservation. Making an assessment of the distribution and conservation status of any species first requires that the species can be delimited and recognised. If the unit to be conserved is in a taxonomically complex group, there can be major problems in assessing threats, devising conservation strategies and monitoring their success. As taxonomically complex groups account for almost half of the species on the UK Biodiversity Action Plan 'short list', there are real difficulties in implementing conservation actions for these species (Hollingsworth 2003).

The association between endemism and taxonomic complexity in the British flora is at least partly attributable to recent ice ages. The vast majority of species in the British flora have achieved their current distributions via recent post-glacial colonisation. The limited time period since colonisation (within the last 12,000 years) has given little time for the evolution of endemic species. Those endemic species that are recognised within the British flora typically show a mechanism, or combination of mechanisms that promote rapid biological diversification: these include polyploidy, hybridisation, self-pollination, agamospermy and clonal growth (Stace 1989, 1997). In a recent review of the history of the North Atlantic biota, Brochmann *et al.* (2003) noted that among the 43 hardy vascular plant species accepted as being endemic to the region, there was not a single sexual diploid indicative of long-term evolution. Similarly, mechanisms which promote rapid diversification have been central to the evolution of the endemic element in the British flora; this rapid diversification also results in taxonomic complexity and knock-on problems for conservation.

MECHANISMS UNDERLYING TAXONOMIC COMPLEXITY

One mechanism that promotes rapid diversification and also leads to taxonomic complexity is a change in breeding system. The evolution of self-pollination (autogamy) from outcrossing (allogamy) is one of the most frequent evolutionary transitions in flowering plants (Stebbins 1974). This change can lead to a neospecies achieving rapid reproductive isolation from its progenitor (Levin 2000), and can generate morphological differences between selfers and their allogamous progenitors due to an increased level of homozygosity. Morpho-

logical characters that include phenotypes encoded by recessive alleles can be selected for, as self-pollination can lead to the fixation of advantageous recessive mutations (Charlesworth 1992, Levin 2000). As the age of the self-pollinating lineage increases, novel mutations can result in further morphological differences.

Self-pollinating species typically show higher levels of variation between populations than do outcrossing species (Hamrick & Godt 1996, Nybom 2004). This is attributable to reduced opportunities for gene flow and also potentially an association between self-pollination and short-lived and/or colonist populations, which show rapid differentiation as a consequence of repeated founder events and genetic drift. There is thus the opportunity for morphological divergence between populations within a selfing species. In addition, there is the possibility that outcrossing progenitor species may generate multiple independent selfing lineages of morphologically similar appearance. Both of these scenarios yield sets of populations separated only by subtle morphological differences which can result in difficulties in the allocation of the most appropriate taxonomic rank and affiliation.

TAXONOMIC COMPLEXITY IN *EPIPACTIS*

The genus *Epipactis* is a classic example of a plant group in which active diversification and breeding system transitions appear to have led to taxonomic complexity and subsequent problems for conservation. The genus consists of between 25 and 60 species. They exhibit a predominantly Eurasian distribution with outlying species in North America and North Africa (Bateman *et al.* 2005). The taxonomic complexity in the genus is reflected in the uncertainty regarding species numbers, and for instance the number of European species accepted by Delforge changed from 36 to 58 in seven years (cf. Delforge 1995, 2001), whereas Sundermann (1970) recognised only 14 species.

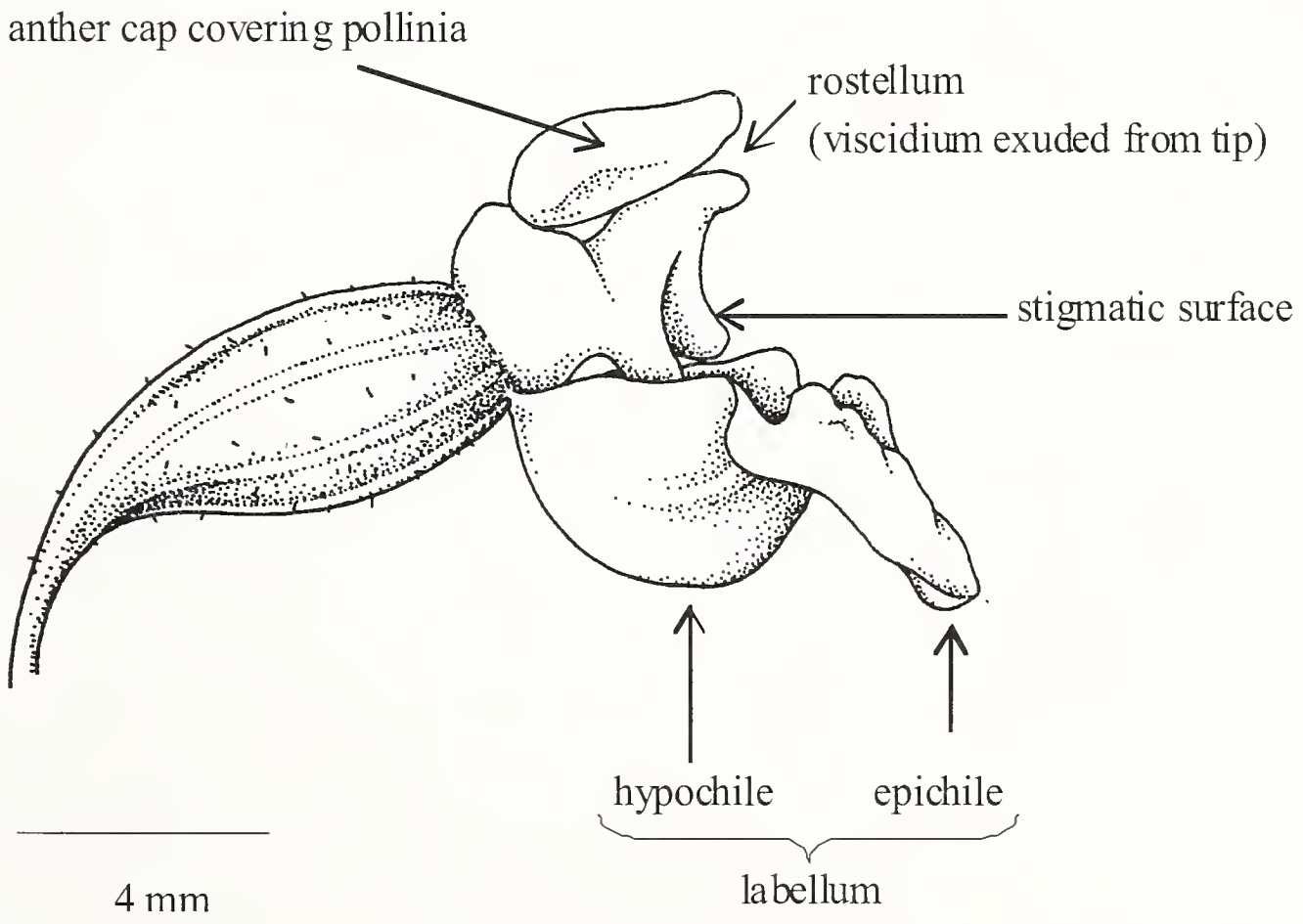


FIGURE 1. Floral morphology of an allogamous *Epipactis* flower. Drawing by Mary Mendum

The floral morphology of species in the genus varies considerably. The two major informal groups in the genus are the '*palustris*' group (rhizomatous clonal species, with an open concave hypochile) and the 'helleborine' group which are typically more vegetatively discrete and possess a more cupped hypochile. The 'helleborine' group (the subject of this paper) encompasses two contrasting floral morphologies. Many species, including the widespread *E. helleborine* have a well-developed rostellum that serves as a barrier between the male and female parts of the flower (Fig. 1). The viscidium is exuded from the tip of the rostellum (Richards 1982) and serves as a glue to bind the pollinia to visiting insects. The vast majority of taxonomic problems in the genus are centred around a large number of named taxa in which the viscidium and rostellum are strongly reduced or are less persistent. Any reduction in the rostellum diminishes the physical barrier between the male and female parts of the flower, while any reduction in the viscidium lowers the likelihood of the pollinia being transferred to flowers on a different plant. Instead, the pollinia can remain *in situ* and fall downward onto the stigmatic surface, resulting in self-pollination. A series of changes such as a reduction in flower size, a more pendulous habit, and reduction in pigmentation have been invoked as secondary modifications which further promote self-pollination (Richards 1982).

Taxa with a floral morphology consistent with self-pollination are typically of restricted distribution, and can show limited variation within populations but subtle and consistent morphological differences between populations. At the heart of taxonomic complexity in *Epipactis* is the extent to which populations with floral morphologies such as these reflect minor mutational variants of single self-pollinating lineages, or whether they reflect independent taxa resulting from iterative allogamy-to-autogamy transitions.

In Britain seven species were recognised by Stace (1997). Four of these have an outcrossing type of floral morphology: *E. palustris, E. atrorubens, E. helleborine* and *E. purpurata* and three have a floral morphology consistent with autogamy: *E. phyllanthes*, *E. leptochila* and *E. youngiana.* Molecular data have shown that within the variation encompassed by *E. leptochila* there are two clearly distinct taxa best treated separately as *E. dunensis* (T. Stephenson & T.A. Stephenson) Godfery and *E. leptochila* s.s. (Godfery) Godfery, and also a third lineage endemic to Lindisfarne (Squirrell *et al.* 2002) which has subsequently been named *E. sancta* (P. Delforge) P. Delforge (Delforge & Gévaudan 2002; Bateman 2006).

EPIPACTIS YOUNGIANA AS A CASE STUDY

One British *Epipactis* species that remains enigmatic is the high conservation profile endemic *E. youngiana* A. J. Richards & D. F. Porter. This species was first described in 1982 and was found growing on some mine spoil heaps in Northumberland (Richards & Porter 1982). It has subsequently been recorded from some similar habitats in Scotland, centred around Glasgow and Falkirk (Dickson *et al.* 2000). It has a floral morphology consistent with self-pollination; in particular its viscidium withers very rapidly. Its presence predominantly in anthropogenic sites has led to suggestions of a recent evolutionary origin stemming from either (a) divergence from *E. helleborine* or (b) a hybrid origin between *E. helleborine* and *E. dunensis*. Self-pollination is considered to have led to rapid reproductive isolation from other *Epipactis* species. As an endemic British orchid species (one of very few endemic plant species recognised in Britain), *E. youngiana* has been afforded full conservation status under Schedule 8 of the Wildlife and Countryside Act (1981). The species also has a Species Action Plan as part of the UK Biodiversity Action plans (UK Biodiversity Group 1995), and associated allocation of conservation resources. However, populations of *E. youngiana* occur

sympatrically with plants of *E. helleborine* (and also at some sites with either *E. phyllanthes* or plants resembling *E. dunensis*). The mixture of taxa growing together and the presence of plants of intermediate morphologies has caused difficulties in identification of material and uncertainty over the taxonomic status of *E. youngiana.* Thus *E. youngiana* receives formal conservation protection, but conservation actions are extremely difficult to implement due to taxonomic uncertainty.

To provide some insights into the taxonomic distinctness of *E. youngiana*, and more generally into the evolutionary processes underlying taxonomic complexity in *Epipactis,* we have carried out large-scale genetic surveys using a combination of sequencing of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and the chloroplast *trn*L intron, allozyme analyses, and screening of RFLP variation and mononucleotide length variation in the *trn*L intron. The full details of this work will be published elsewhere; our goals here are to explore the taxonomic status of *E. youngiana* and to infer the most appropriate conservation treatment of this putative species.

MATERIALS AND METHODS

A total of 2828 individuals of 164 populations of 23 named species in the 'helleborine' group have been examined using a range of genetic markers (Table 1). The species were selected to encompass a range of putative species including several with a floral morphology consistent with self-pollination and several with a floral morphology consistent with outcrossing. The sampling of *E. youngiana* involved two populations from Northumberland and two from Scotland. Sympatric plants of *E. helleborine* (and *E. dunensis* and *E. phyllanthes* where present) were also sampled from these sites.

These samples have been examined using a range of molecular approaches (allozyme electrophoresis, chloroplast and ITS sequencing, chloroplast microsatellites and RFLPs). Between nine and ten allozyme loci were screened, eight of which were polymorphic. At the polymorphic loci there were between 2-4 alleles. The chloroplast data set considered here consists of information from the *trn*L intron. Based on sequences of 86 individuals, two variable markers were selected for widescale screening. Firstly, we screened for the presence or absence of a 10 bp duplication in the *trn*L intron via RFLPs, and secondly we examined length polymorphism in a chloroplast microsatellite that consisted of a mononucleotide poly-A repeat of between $(A)_9$ and $(A)_{13}$ bp. Sequencing of the nuclear ITS regions from 63 individuals revealed phylogenetic resolution at deeper levels within the genus, but within the taxonomically complex *E. helleborine* group little variation was detected. Consequently information from ITS is not discussed further in this paper.

RESULTS AND DISCUSSION

PATTERNS OF ALLOZYME VARIATION

The *Epipactis* species examined here show some marked differences in the amounts and organisation of genetic variation among individuals and populations (Table 2). With the exception of plants from the *E. youngiana* sites (which are discussed later), all of the species examined with a floral morphology consistent with self-pollination consisted of homozygous and genetically uniform lines. No heterozygous individuals were detected in the total dataset

TABLE 1. *EPIPACTIS* TAXA SAMPLED, DISTRIBUTIONS, FLORAL MORPHOLOGY, SAMPLE LOCATIONS, AND SAMPLE SIZES FOR DIFFERENT MOLECULAR ASSAYS. Floral morphology designation and geographical range is based on field observations and Delforge (2001). n = sample size for different assays.

Species	Floral morphology	Distribution	Countries sampled	n pops	n Allo-zymes	n ITS	n *trn*L	n duplication	n cp SSR
Epipactis albensis	Autogamous	Germany/Austria	Austria	1	2	1	1	1	1
Epipactis atrorubens	Allogamous	Eurasia	England, Scotland, Wales, France, Norway	9	162	1	1	26	48
Epipactis campeadorii	Autogamous	Spain	Spain	1	5	1	1	5	1
Epipactis confusa	Autogamous	Scandinavia/Germany	Denmark	1	20	1	1	18	11
Epipactis distans	Allogamous	European Alpine	France	1	20	2	2	6	16
Epipactis dunensis	Autogamous	UK	England, Scotland, Wales	8	171	15	14	130	108
Epipactis fibri	Autogamous	France	France	1	21	1	1	18	1
Epipactis futakii	Autogamous	Slovakia	Slovakia	1	3	1	1	2	1
Epipactis helleborine	Allogamous	Eurasia (introduced N. America)	England, Scotland, Denmark, France, Belgium, Canada, Switzerland, Germany	47	1117	10	10	865	746
Epipactis leptochila	Autogamous	Europe	England, France, Germany	18	234	5	5	162	131
Epipactis microphylla	Autogamous	Eurocaucasia	France, Switzerland, Germany	7	72	1	1	53	9
Epipactis muelleri	Autogamous	Mid-Europe	France, Germany	7	77	4	4	62	72
Epipactis parviflora	Allogamous	Spain/France	Spain, France	2	10	1	1	8	0
Epipactis peitzii	Autogamous	Germany	Germany	1	3	1	1	6	1
Epipactis phyllanthes	Autogamous	Atlantic Europe	England, France, Switzerland, Germany, Ireland, Spain	27	430	13	13	307	157
Epipactis placentina	Autogamous	Central Europe	France, Slovakia	2	1	2	2	3	17
Epipactis provincialis	Autogamous	France	France	1	22	3	3	22	22
Epipactis pseudopurpurata	Autogamous	Eastern Europe	Slovakia	1	4	0	0	1	0
Epipactis purpurata	Allogamous	Temperate Europe	England, Denmark, France, Germany	18	207	3	3	23	79
Epipactis rhodanensis	Autogamous	France/Switzerland	France, Switzerland	4	96	4	4	67	33
Epipactis sancta	Autogamous	England	England	1	26	4	4	26	16
Epipactis tremolsii	Allogamous	Mediterranean	France	1	16	0	0	16	0
Epipactis youngiana	Autogamous	UK	England, Scotland	4	89	2	2	64	92

for the examined loci. Although this uniformity precludes formal breeding system estimates, it is at the very least strongly congruent with self-pollination leading to homozygosity and uniformity. In contrast, all of the species with a floral morphology consistent with outcrossing exhibited at least some genetic variation and some heterozygosity (Table 2). The inbreeding coefficient in all of these species was not significantly different from zero, and thus was consistent with random mating. The one minor exception was *E. atrorubens*; despite heterozygous individuals being detected, there was a small but significant deficit of heterozygotes compared with Hardy-Weinberg expectations (F_{IS} = 0.180; Table 3). It is not clear why this species should show some slight heterozygote deficit when the other allogamous species do not. Possible explanations are that these populations experience higher levels of geitonogamy or lower pollinator availability than the other species. However, aside from this minor exception, there is a marked correlation between floral morphology and patterns of genetic variation, and these results confirm the importance of minor modifications in floral morphology as determinants of the organisation of genetic variation (Richards 1982).

However, floral morphology is not the only determinant of patterns of allozyme variation in these species. Although there is homogeneity in the variation patterns within the putative self-pollinating taxa (all taxa show zero within-species variation for these loci), the situation is more varied among the allogams (Table 3). Levels of variation ranged from 11% to 56% of allozyme loci being variable within populations, and although all populations of most species showed some variation, in *E. purpurata* only 47% of sampled populations were variable (Table 3). This suggests that other factors, such as population size, plant size, demographic history and pollinator activity are also likely to be important determinants of the amounts and partitioning of genetic variation.

Considering the range of allelic variation between the species with outcrossing and selfing floral morphologies, for the most part the different selfing lineages are fixed for different character combinations that represent subsets of the alleles found in the allogams (Table 2). This pattern is consistent with multiple transitions from outcrossing to selfing, giving rise to a series of genetically and morphologically discrete lineages, rather than the taxonomic complexity in the genus stemming from a single outcrossing-selfing transition followed by mutational divergence. Thus the transition from allogamy to autogamy seems particularly labile in *Epipactis*. Of course, multiple origins of selfing and mutational divergence of lineages are not mutually exclusive and following independent origins there is the possibility for further divergence. Although this aspect of the work is beyond the scope of this paper and will be explored elsewhere, it is worth noting that there is evidence for some alleles present in autogams that have not been found in allogams, and also for between-population divergence in autogamous taxa (Table 2). For example, the inland populations of *E. dunensis* differ genetically from the coastal populations (Table 2; see also Squirrell *et al.* 2002) and there are further differences between populations of *E. microphylla* for the rapidly evolving cpSSRs locus (Table 2).

In summary, genetic variability and heterozygosity are found within putative outcrossing taxa (albeit with differences in absolute levels of diversity). In contrast, the autogamous lineages are united in their allozyme uniformity and complete lack of within-population variation. How does the British endemic *E. youngiana* match these patterns of variability, and does the evidence support the notion that it is a distinct, recently evolved species that has achieved reproductive isolation via the evolution of autogamy?

TABLE 2. THE DISTRIBUTION OF ALLOZYME AND CHLOROPLAST VARIANTS WITHIN EUROPEAN *EPIPACTIS* SPECIES. (See top of following page for details)

*Epipactis s*pecies	Allozyme loci								Chloroplast (*trn*L)	
Autogams	mdh-1	mdh-2	idh-1	pgm	aat-1	pgi-1	pgd	idh-2	Duplication present	SSR no. repeats
confusa[1]	c	c	a	c	a	b	a	a	No	9
phyllanthes[1]	c	c	a	c	a	b	a	a	No	9
albensis	b	a	a	a	a	c	a		Yes	10
campeadorii[2]	b	c	a	b	a	c	a		No	10
dunensis (w coastal)	b	c	a	b	a	c	a		Yes	11
dunensis (inland)	b	c	a	b	a	c	a		Yes	10
fibri	b	b	a	a	c	a	a		Yes	10
futakii	b	b	a	a	a	c	a		No	9
leptochila	b	b	a	a	a	c	a		No	11
microphylla	a	d	a	a	a	b	a		No	12/13[3]
muelleri	a	b	b	a	b	b	a		No	10
peitzii	a	b	a	a	a	c	a		Yes	12
placentina	a	b	a	a	b	b	a		Yes	9
provincialis	b	b	a	a	a	a	a		No	11
pseudopurpurata	a	b	a	a	b	a	a		No	
rhodanensis	b	c	a	a	a	c	a		No	11
sancta[2]	b	c	a	b	a	c	a		No	10
Allogams										
atrorubens	ab	abc	acd	abc	abc	abc	a		No	9
distans	bc	b	a	a	a	c	a		No	11
helleborine	ab	abc	abc	ab	abcd	abc	ab		No & Yes	9/10/11
parviflora	a	ab	ad	a	a	ab	a		No	
purpurata	ab	bc	ab	ab	abc	abc	a		Yes	10/11
tremolsii	ab	abc	abd	ab	abc	abc	a		No	10
Settlingstones										
helleborine	ab	abc	a	ab	abc	abc	a		No & Yes	10/11
youngiana	ab	abc	a	ab	abc	abc	a		No & Yes	10/11
phyllanthes	a	c	a	c	a	b	a	a	No	9
Bardykes Bing										
helleborine	ab	bc	ab	ab	abc	abc	a		Yes	9/10
youngiana	ab	bc	ab	ab	abc	abc	a		No & Yes	9/10
Bothwell Castle										
helleborine	ab	bc	ab	ab	abc	abc	a		No & Yes	10/11
youngiana	ab	bc	ab	ab	ab	abc	a		No & Yes	9/10/11
dunenesis[4]	ab	bc	ab	ab	a	bc	a		Yes	10/11
Gosforth Park										
helleborine	ab	abc	a	ab	ab	abc	a		No & Yes	10/11
youngiana	ab	abc	a	ab	a	abc	a		No & Yes	10/11

Table 2 cont. The different letters represent the allelic variants found within each taxon. [1]Our genetic data were unable to distinguish between *E. confusa* and *E. phyllanthes*; the taxonomic implications of this will be discussed elsewhere. [2]The data presented here do not distinguish *E. sancta* and *E. campeadorii*[2] but these two species can be distinguished based on their ITS sequences (data not shown). [3]Different populations of *E. microphylla* are fixed for either 12 or 13 repeats at the cpSSR locus. [4]Plants with the morphology of *E. dunensis* at the *E. youngiana* sites in Scotland do not show the classic homozygous and uniform *E. dunensis* allozyme profile.

TABLE 3. WITHIN-POPULATION DIVERSITY MEASURES AND ESTIMATES OF THE INBREEDING COEFFICIENT IN ALLOGAMOUS EUROPEAN *EPIPACTIS* SPECIES BASED ON NINE ALLOZYME LOCI

Species	N pops	Mean n	*P*	*A*	F_{IS}	H_E	*PP*
E. atrorubens	9	17.8	29	1.37	0.180*	0.120	100
E. distans	1	19.0	11	1.11	0.390^{ns}	0.038	100
E. parviflora	2	5.0	12	1.12	-0.148^{ns}	0.056	100
E. purpurata	18	11.5	11	1.12	0.033^{ns}	0.024	47
E. tremolsii	1	15.2	56	2.00	0.009^{ns}	0.227	100
E. helleborine	47	23.5	56	1.81	0.003^{ns}	0.231	100

N pops = number of populations; Mean n = mean sample size per population per locus; *P* = % polymorphic loci; *A* = mean number of alleles per locus; F_{IS} = global inbreeding coefficient; * = significantly different from zero $p < 0.05$, ^{ns} = not significant; H_E = gene diversity; *PP* = % of populations that are polymorphic.

TABLE 4. WITHIN-POPULATION DIVERSITY MEASURES AND ESTIMATES OF THE INBREEDING COEFFICIENT IN FOUR SYMPATRIC POPULATIONS OF *E. YOUNGIANA* AND *E. HELLEBORINE* IN BRITAIN BASED ON NINE ALLOZYME LOCI

Species	Region	Location	n	*P*	*A*	F_{IS}
E. helleborine	Northumberland	Settlingstones	30	56	1.89	0.110^{ns}
	Northumberland	Gosforth Park	9	56	1.78	0.139^{ns}
	Glasgow	Bardykes Bing	52	67	1.89	0.035^{ns}
	Glasgow	Bothwell Castle	31	67	1.89	-0.059^{ns}
E. youngiana	Northumberland	Settlingstones	36	56	1.89	0.036^{ns}
	Northumberland	Gosforth Park	8	44	1.67	0.099^{ns}
	Glasgow	Bardykes Bing	21	67	1.89	0.141*
	Glasgow	Bothwell Castle	24	67	1.78	-0.048^{ns}

n = sample size, *P* = % polymorphic loci, *A* = mean number of alleles per locus, F_{IS} = inbreeding coefficient, * = significantly different from zero $p < 0.05$.

IS *E. YOUNGIANA* AUTOGAMOUS? (NO)

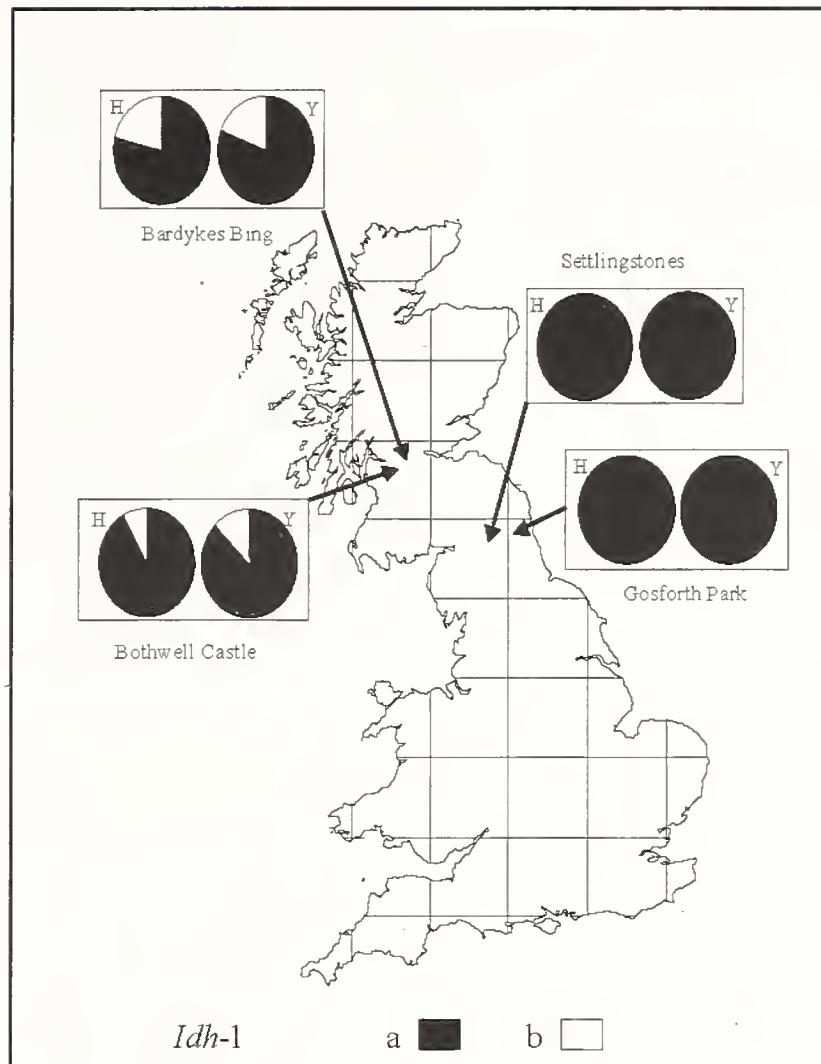
E. youngiana typically occurs in sympatry with *E. helleborine* and sometimes also with other *Epipactis* species. The mechanism proposed for the development of reproductive isolation and speciation is self-pollination. There is, however, no clear evidence that the plants ascribed to *E. youngiana* are undergoing self-pollination. Both our studies and those of Harris & Abbott (1997) have recovered high levels of heterozygosity in populations of this species. In three of the four populations examined here, the distribution of alleles among individuals is consistent with random sexual mating (Table 4). Only in the Bardykes Bing population was a (just) significant deviation from Hardy-Weinberg equilibrium detected ($p = 0.05$), and this was attributable to a very minor deficit of heterozygosity that might represent a sampling artefact (an allozyme survey of the same population by Harris & Abbott (1997) did not detect any significant deviation from Hardy-Weinberg expectations). If complete self-pollination was occurring, the rate of homozygosity should increase by 50% per generation. Even allowing for non-overlapping generations and a recent origin for the species, if self-pollination had been sufficiently extensive to lead to reproductive isolation from sympatric plants of *E. helleborine,* it would be expected to leave a much stronger signature on the partitioning of allelic variation within and among individuals.

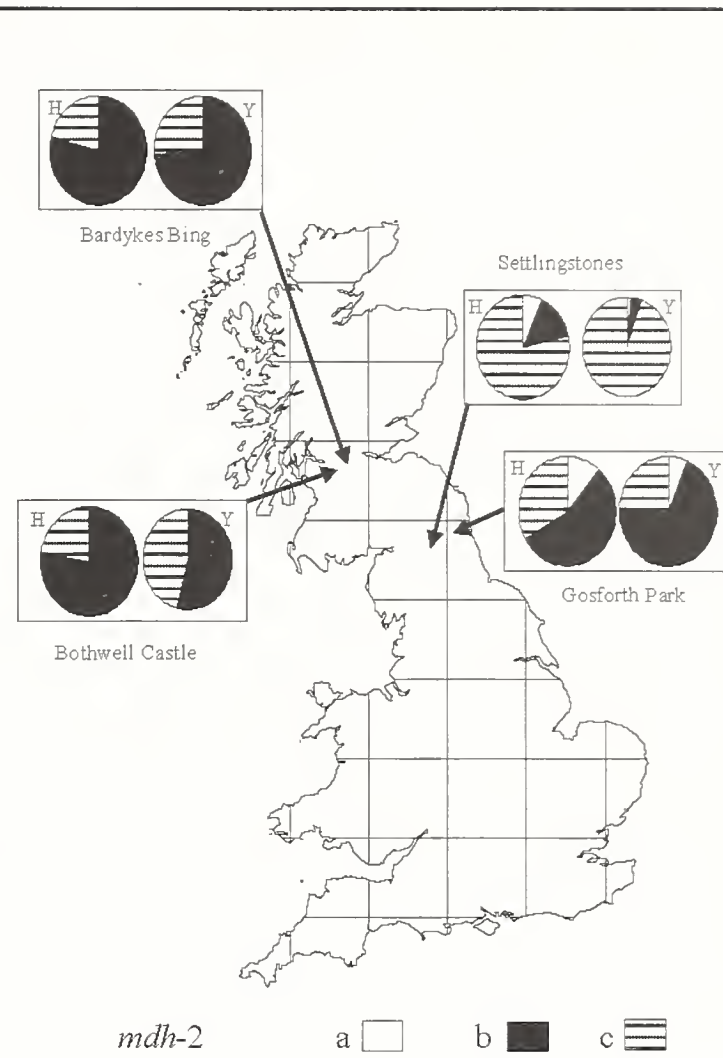
IS *E. YOUNGIANA* DISTINCT? (NO)

If *E. youngiana* is a distinct cohesive species, we should expect allele frequencies in different populations of *E. youngiana* to be more similar to one another than to local populations of *E. helleborine.* However, this is not the pattern recovered from either the allozyme data or the cpDNA data. For both chloroplast and nuclear allozyme data, the allelic diversity in *E. youngiana* and *E. helleborine* shows greater similarities by site than by taxon (Figs 2, 3). Indeed, if one tests for random mating by pooling individuals of *E. helleborine* and *E. youngiana* at each site, at three of the four sites no significant deviation from random mating is detected among individuals *between* 'species' ($F_{IS} = 0.062$ Bardykes Bing, $F_{IS} = -0.036$ Bothwell Castle, $F_{IS} = 0.172$ Gosforth Park; all non-significant). In the fourth population (Settlingstones), pooling individuals between *E. helleborine* and *E. youngiana* does result in a statistically significant departure from random mating, but the deviation from panmixia is again minor ($F_{IS} = 0.091$, $p = 0.043$).

ARE POPULATIONS OF *E. YOUNGIANA* NORMAL POPULATIONS OF *E. HELLEBORINE* THAT HAVE BEEN TAXONOMICALLY OVER-SPLIT? (NO)

Even the briefest of visits to the populations of *E. youngiana* in both Northumberland and Scotland reveals a pattern of morphological diversity outwith the norm. The classic '*youngiana*' morphology is not a phenotype that is present in typical populations of *E. helleborine*. There is undoubtedly something unusual about these populations which contain atypical mixtures of floral morphologies, ranging from individuals whose floral morphology resembles autogamous plants, to those whose floral morphology resembles outcrossing plants. Given that our data suggests that self-pollination has





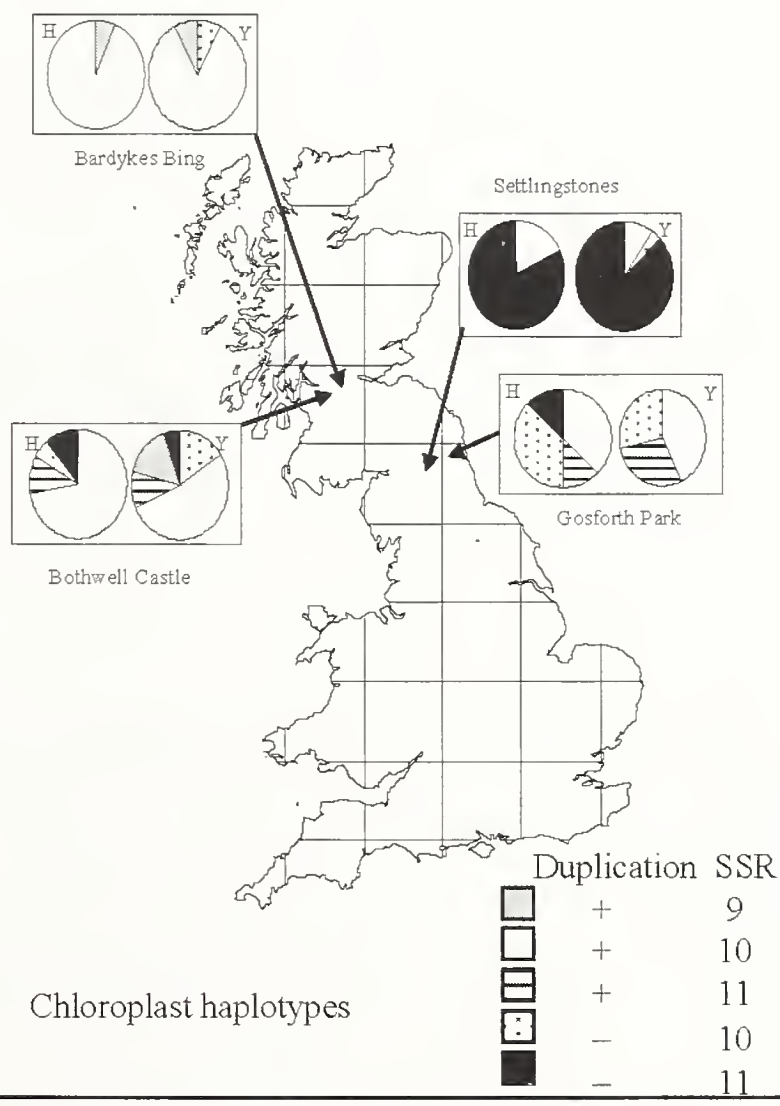


Figure 2. The distribution of chloroplast haplotype variation and allelic variants for two allozyme loci in four sympatric populations of *E. youngiana* and *E. helleborine* in Britain. If *E. youngiana* was a distinct species, populations of this taxon should be more genetically similar than they are to sympatric populations of *E. helleborine*. Instead, greater generic similarities are found between *E. youngiana* and the local *E. helleborine* populations.

H = *E. helleborine*,
Y = *E. youngiana*

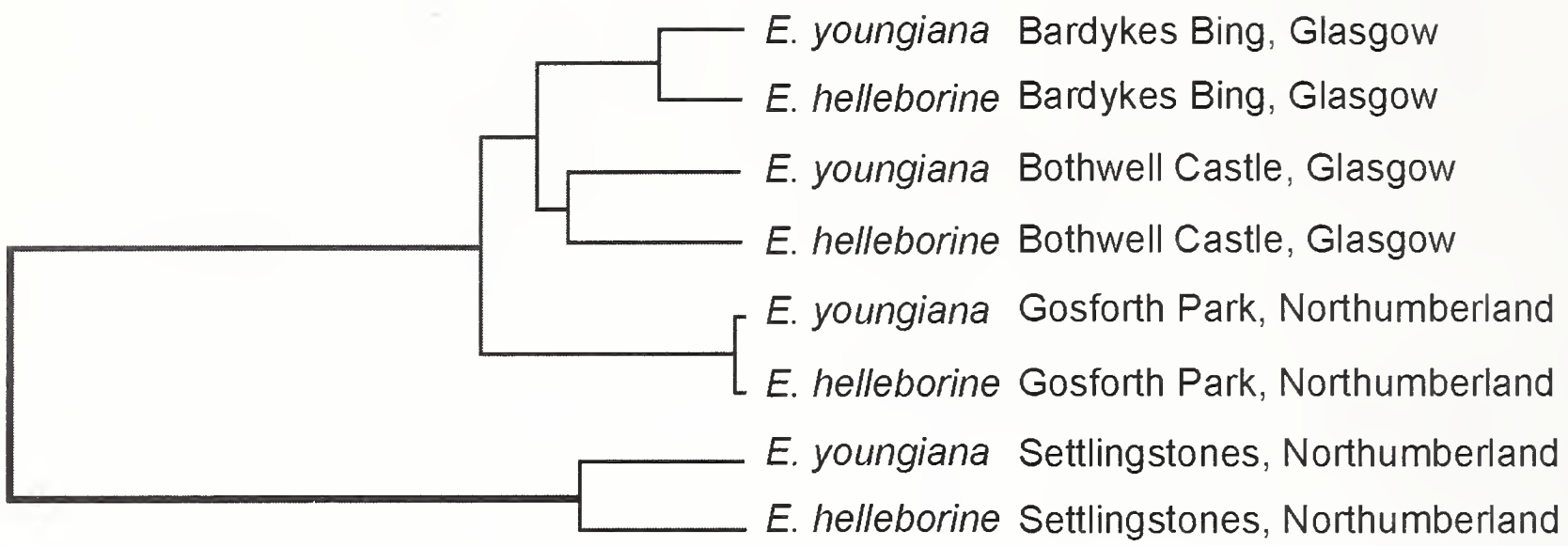


FIGURE 3. UPGMA clustering based on pairwise estimates of population differentiation (F_{ST}) for eight polymorphic allozyme loci from four sympatric populations of *E. youngiana* and *E. helleborine* in Britain. Populations of *E. youngiana* do not form a distinct cohesive genetic entity; instead, each population is more closely related to its local *E. helleborine* population.

arisen from outcrossing taxa on numerous occasions in *Epipactis*, perhaps it is not surprising to find populations in which there is a mixture of floral morphologies, even if there is as yet no clear divergence of taxa. It is possible that these polymorphic populations represent the type of population from which future autogamous lineages may originate.

The apparent absence of self-pollination in *E. youngiana*, despite the flowers having a selfing floral morphology, may reflect the presence of sympatric allogamous *E. helleborine* at these sites. Even if pollinia export is reduced in individuals with the *E. youngiana* morphology, these plants presumably can behave as functional females and receive pollinia import from neighbouring allogamous *E. helleborine* plants via visiting pollinators. To achieve reproductive isolation, plants with a selfing floral morphology may need to disperse to a site where no allogamous plants occur.

ARE THE *E. YOUNGIANA* POPULATIONS TYPICAL OF TAXONOMIC COMPLEXITY IN *EPIPACTIS*? (NO)

The populations of *E. youngiana* show a very different pattern of genetic variation from the consistent pattern seen in autogamous *Epipactis* species (all of which were homozygous and uniform for the allozyme loci considered here; Figure 4b). There is thus a clear difference between *E. youngiana* and the vast majority of other autogamous lineages recognised at the species level in *Epipactis* (Figs 4a, b; Table 2). The only close parallel we are aware of is *E. renzii* Robatsch, a taxon restricted to coastal dunes in Denmark. Based on allozyme electrophoresis, Pedersen & Ehlers (2000) concluded that it had arisen on multiple occasions from local populations of *E. helleborine* subsp. *neerlandica* (Verm.) Buttler. Like *E. youngiana*, *E. renzii* occurs sympatrically with populations of *E. helleborine* and at individual sites

the two putative taxa share the same alleles. Unlike *E. youngiana,* all three populations of *E. renzii* examined by Pedersen & Ehlers (2000) showed a strongly significant inbreeding coefficient ($F_{IS} = 0.486$, $F_{IS} = 0.832$, $F_{IS} = 1.0$, all $p < 0.001$). The authors concluded that the origin of self-pollination was recent and attributable to adaptation for reproductive assurance due to a short flowering season caused by water stress and early wilting of flowers. Based on the absence of extensive molecular and morphological divergence, Pedersen & Ehlers (2000) argued that *E. renzii* should be given varietal status rather than species status. This apparently intermediate phase represented by *E. renzii,* between allogamous populations of *E. helleborine* and homozygous uniform autogamous segregates is interesting, and a parallel taxonomic treatment for *E. youngiana* (varietal status) may be appropriate. This approach was adopted informally by Lang (2004) and Bateman (2006), and a formal transfer was performed by Kreutz (2004).

WHAT IS THE MECHANISM FOR THE RECURRENT EVOLUTION OF A SELFING FLORAL MORPHOLOGY WITHIN *EPIPACTIS*?

This assessment of patterns of genetic diversity in *Epipactis* has demonstrated the frequency of transitions in floral morphology from outcrossing to selfing types. Given the lability of this switch, it is worth evaluating the evolutionary processes hypothesised to underlie these transitions. Selection for reproductive assurance under conditions of poor pollinator availability and/or a short flowering season provide some explanations for the advantages of self-pollination, but not the mechanism underlying the transitions. Pedersen & Ehlers (2000) argued that recurrent mutations may be responsible and noted that a mutation resulting in paedomorphosis via an arrested development of the rostellum may be the key step required in the evolution of autogamy. Whilst we consider this hypothesis plausible, we also believe that an alternative hypothesis is worth considering: that hybridisation between autogamous and allogamous *Epipactis* species provides a mechanism for the transfer of genes encoding the selfing-floral morphology into novel heterozygous backgrounds from which new selfing lineages with new character combinations can arise. Under this scenario the evolution of autogamy could in some cases be considered as a cyclical process more akin to an 'evolutionary detour' than the 'evolutionary dead-end' proposed by Stebbins (1957). Selfing lineages evolve and differentiate, and at some future point occur in sympatry with allogamous taxa, hybridise and result in the production of further selfing lineages.

As yet there is little evidence to support or refute this 'evolutionary detour' hypothesis. Evidence from mixed populations of autogamous taxa (e.g. mixed populations of *E. phyllanthes* and *E. dunensis* on the west coast of England) suggests that these taxa co-exist at high densities without undergoing any gene exchange (Fig. 5). However, where we have sampled populations of putative autogams occurring in sympatry with allogamous taxa in the complex *E. youngiana* sites, the pattern changes somewhat:

1 Plants with a morphology consistent with *E. dunensis* occur in the same complex sites that contain Scottish populations of *E. youngiana* and *E. helleborine*. At these sites, plants with the morphology of *E. dunensis* are genetically variable and heterozygous for the same alleles found in local populations of *E. helleborine* and *E. youngiana* (Fig. 4, Table 2), and plants of intermediate morphologies occur. This is a marked contrast with

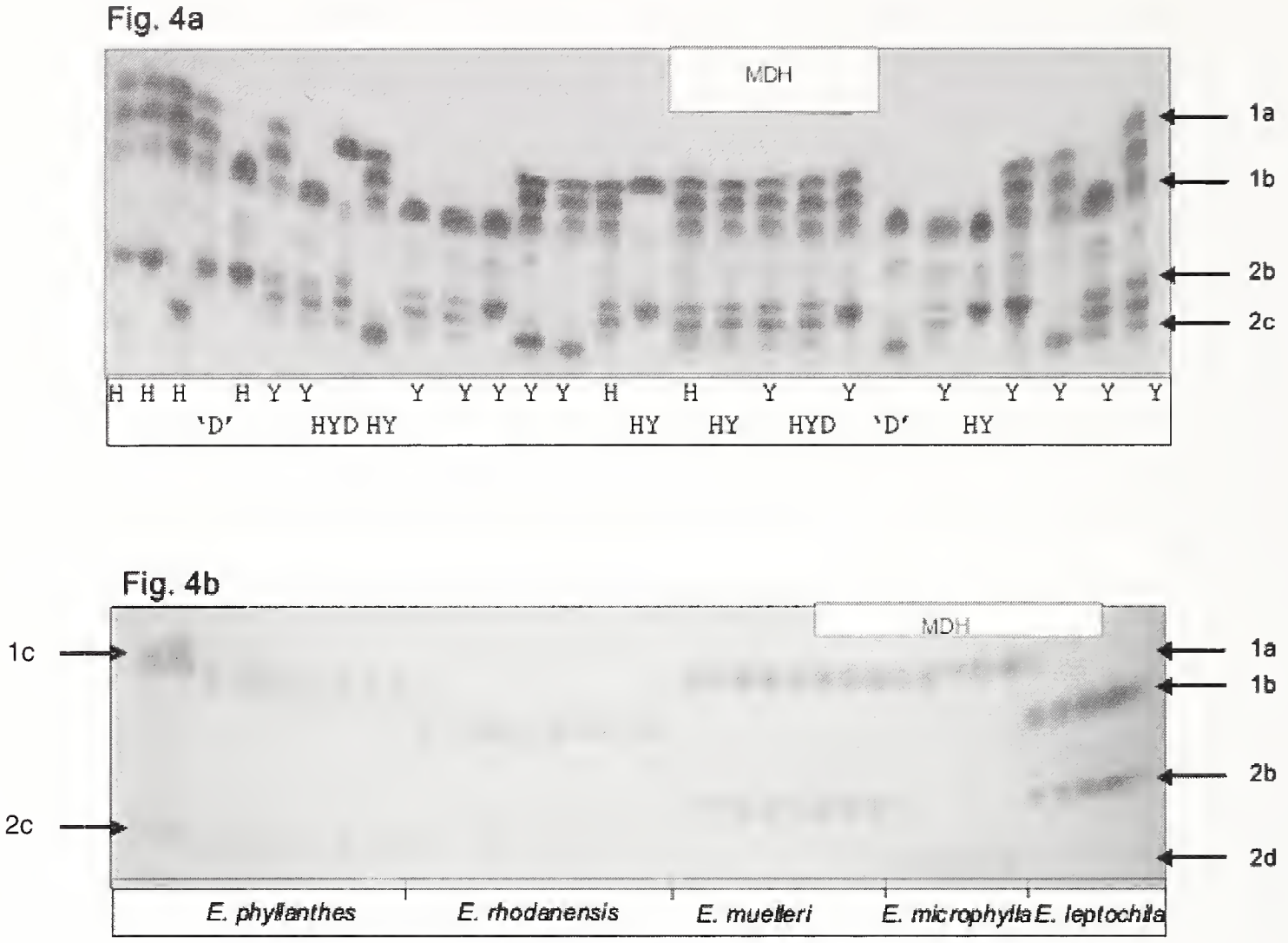


FIGURE 4. Patterns of allozyme diversity in a complex *E. youngiana* population compared with typical autogamous *Epipactis* taxa. (a) MDH variation at two loci from a mixed Scottish population (Bothwell Castle) of plants with the morphology of *E. youngiana*, *E. helleborine* and *E. dunensis* showing high levels of heterozygosity for the same set of alleles; H = *E. helleborine*, Y = *E. youngiana*, and 'D' = plants which resemble morphologically *E. dunensis* (no plants with the classic *E. dunensis* molecular genotype have yet been detected in Scotland). Combinations of these letters represent morphological intermediates. (b) MDH variation at two loci showing the classic autogamous genetic signature in *Epipactis*: fixed homozygous and uniform allozyme genotypes within species, but fixed differences for different allelic combinations between species.

Arrows represent locus (number) and allele (letter) designations as used in Table 2. Unlabelled bands were not scored and are assumed to represent heterodimers and breakdown products.

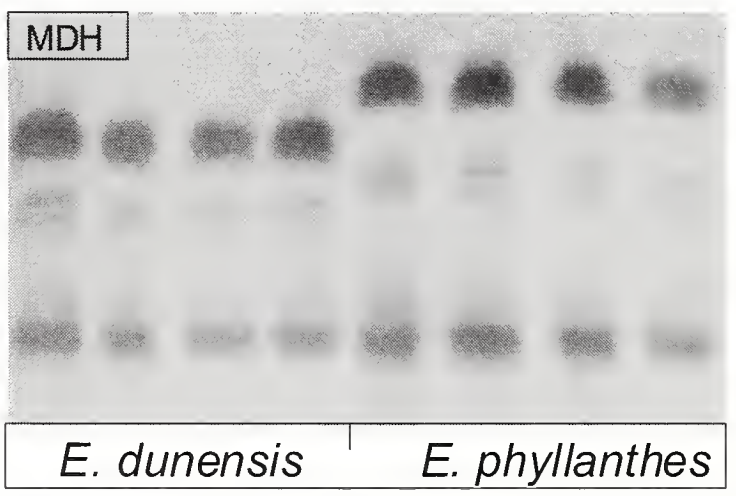


FIGURE 5. Representative MDH variation in plants of *E. dunensis* and *E. phyllanthes* from a mixed site on the north-west coast of England, demonstrating the maintenance of clear genetic differences between autogamous species growing in sympatry.

all other *E. dunensis* populations we have examined, in which the plants are all genetically distinct, homozygous and uniform, and suggests that the plants of *E. dunensis*-type morphology in the Scottish sites are not 'pure'.

2 Likewise, at Settlingstones, the local plants of *E. phyllanthes* possess an MDH allele that is also found in the local *E. helleborine* and *E. youngiana* populations. This was absent from a survey of 408 plants from 26 other populations of *E. phyllanthes*, all of which showed a single uniform allozyme profile. This again may indicate some past hybridisation, although *E. phyllanthes* at this site appears to be morphologically uniform and typical.

3 One additional curious feature of the Settlingstones site is that for both *E. helleborine* and *E. youngiana* there is a high frequency of an unusual chloroplast type (82% and 87% respectively: Fig. 2). Although present at similar frequencies in some populations of *E. helleborine* introduced to North American (Squirrell *et al.* 2001) this haplotype is typically absent or occurs at a low frequency in British populations (in a survey of ten populations it ranged from a frequency of zero to 9.5%, mean = 1%). This may just be chance, although the coincidence of this atypical marker in a taxonomically complex population also may suggest a genetic signature of past hybridisation. However, if this pattern is due to hybridity, it is not obvious which species was involved (this haplotype is not found in geographically proximal species such as *E. dunensis* or *E. phyllanthes*). The only other British species that possesses this chloroplast haplotype is *E. leptochila* s.s., a species with a much more southerly distribution in the UK, so a hybrid explanation for the unusually high frequency of this marker would require a rather convoluted scenario.

Thus the evidence for the evolutionary detour hypothesis is somewhat circumstantial and equivocal. Further research on mixed populations of allogamous and autogamous taxa is required to test the importance of hybridisation as a mechanism underlying the recurrent origins of self-pollination. However, it does at least seem plausible that a normally autogamous species such as *E. dunensis* may receive insect visits and pollinia import (cf. Richards 1986) if growing in sympatry with an outcrossing species, and thus potentially can serve as a conduit for the transfer of genes encoding selfing floral morphologies into novel heterozygous backgrounds.

IS A SPECIES-BASED APPROACH TO CONSERVATION APPROPRIATE FOR DEALING WITH DIVERSITY IN TAXONOMICALLY COMPLEX GROUPS?

Under current species-based conservation programmes, the conservation status of *E. youngiana* should be revised. The genetic data and the extreme difficulties of identifying morphological discontinuities in the field all suggest that this does not represent a cohesive, distinct, reproductively isolated species that has stabilised by autogamy. Instead, it is best considered as a series of complex populations that have not currently achieved separate evolutionary trajectories from the sympatric populations of *E. helleborine.* Given the available evidence, it would in practice be exceedingly difficult to enforce the current legislative conservation protection of this 'species' under the Wildlife and Countryside Act.

However, it is equally important to note that casually dismissing the conservation value of complex populations like *E. youngiana* is a simplistic view, and that it typifies a widespread problem regarding the conservation of taxonomically complex groups in the postglacial flora of Britain. Work on *Epipactis* has revealed a range of genetically variable allogamous taxa, a range of uniform homozygous lineages with a floral morphology consist-

ent with selfing, and some populations such as *E. youngiana* that fall someway between the two. This pattern of common variable species, local endemic entities, and morphologically complex populations containing individuals not readily assignable to any discrete taxon is paralleled in other actively diversifying taxonomically complex groups such as *Euphrasia* (French 2004, French *et al.* 2005) and *Sorbus* (Robertson *et al.* 2004). Taxonomic complexity, recent/ongoing diversification and endemism are all tightly associated in the British flora, and indeed in that of the broader North Atlantic region. If diversification is ongoing, one should not expect all diversity to fall conveniently into neat discrete packages. Therefore it seems appropriate to develop conservation strategies that encompass the broad range of diversity and evolutionary processes in these groups, rather than focusing attention and resources entirely on the taxonomic status of a fraction of this diversity. An alternative conservation goal for taxonomically complex groups would be to develop conservation strategies aimed at the diversification process itself, which recognise the value of all elements in the system such as progenitor species, endemics and taxonomically complex sites (Hollingsworth 2003, Ennos *et al.* 2005).

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REFERENCES

BATEMAN, R.M. (2006). How many orchid species are currently native to the British Isles? In: J. BAILEY & G. ELLIS (eds), 2006. *Current taxonomic research on the British & European flora*, pp. 89–110. BSBI, London.

BATEMAN, R.M., HOLLINGSWORTH, P.M., SQUIRRELL, J. & HOLLINGSWORTH, M.L. (2005). Neottieae: Phylogenetics. In: A.M. PRIDGEON, P.J. CRIBB, M.W. CHASE & F.N. RASMUSSEN (eds), *Genera Orchidacearum, volume 4*, Epidendroideae I, pp. 487–495. Oxford University Press, Oxford.

BROCHMANN, C., GABRIELSEN, T.M., NORDAL, I., LANDVIK, J.Y. & ELVEN, R. (2003). Glacial survival or *tabula rasa*? The history of the North Atlantic biota revisited. *Taxon* **52**: 417–450.

CHARLESWORTH, B. (1992). Evolutionary rates in partially self-fertilizing species. *American Naturalist* **140**: 126–148.

CLAPHAM, A.R., TUTIN, T.G. & MOORE, D.M. (1989). *Flora of the British Isles*, edn 3. Cambridge University Press, Cambridge.

DELFORGE, P. (1995). *Orchids of Britain and Europe*. Harper-Collins, London.

DELFORGE, P. (2001). *Guide des orchidées d' Europe*. Delachaux et Niestlé, Lausanne.

DELFORGE, P. & GÉVAUDAN, A. (2002). Contribution taxonomique et nomenclaturale au groupe d' *Epipactis leptochila*. *Les Naturalistes belges, hors-série – spécial Orchidées* **83** (Orchid 15): 19–35.

DICKSON, J.H., MACPHERSON, P. & WATSON, K. (2000). *The changing flora of Glasgow: Urban and rural plants through the centuries*. Edinburgh University Press, Edinburgh.

ENNOS, R.A., FRENCH, G.C. & HOLLINGSWORTH, P.M. (2005). Conserving taxonomic complexity. *Trends in Ecology and Evolution* **20**: 164–168.

FRENCH, G.C. (2004). Conservation genetics of the critical plant genus *Euphrasia* L. Unpublished PhD thesis, University of Edinburgh and the Royal Botanic Garden Edinburgh.

FRENCH, G.C., ENNOS, R.A., SILVERSIDE, A.J. & HOLLINGSWORTH, P.M. (2005). The relationship between flower size, inbreeding coefficient and inferred selfing rate in British *Euphrasia* species. *Heredity* **94**: 44–51.

HAMRICK, J.L. & GODT, M.J.W. (1996). Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London, series B* **351**: 1291–1298.

HARRIS, S.A. & ABBOTT, R.J. (1997). Isozyme analysis of the reported origin of a new hybrid orchid species, *Epipactis youngiana* (Young's helleborine) in the British Isles. *Heredity* **79**: 402–407.

HOLLINGSWORTH, P.M. (2003). Taxonomic complexity, population genetics, and plant conservation in Scotland. *Botanical Journal of Scotland* **55**: 55–63.

KREUTZ, C.A.J. (2004). *Kompendium der Europäischen Orchideen.* Published by the author, Landgraaf.

LANG, D. (2004). *Britain's orchids*. Wild Guides Ltd, Old Basing, Hampshire.

LEVIN, D.A. (2000). *The origin, expansion and demise of plant species*. Oxford University Press, Oxford.

NYBOM, H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13**: 1143–1155.

PEDERSEN, H.Æ. & EHLERS, B.K. (2000). Local evolution of obligate autogamy in *Epipactis helleborine* subsp. *neerlandica* (Orchidaceae). *Plant Systematics and Evolution* **223**: 173–183.

PRESTON, C.D., PEARMAN, D.A. & DINES, T.D. (2002). *New atlas of the British & Irish flora*. Oxford University Press, Oxford.

RICHARDS, A.J. (1982). The influence of minor structural changes in the flower on breeding systems and speciation in *Epipactis* Zinn. (Orchidaceae). In: J.A. Armstrong, J.M. Powell & A.J. Richards (eds), *Pollination and evolution*, 47–53. Royal Botanic Gardens, Sydney.

RICHARDS, A.J. (1986). Cross-pollination by wasps in *Epipactis leptochila* (Godf.) Godf. *s.l.* *Watsonia* **16**: 180–182.

RICHARDS, A.J. & PORTER, A.F. (1982). On the identity of a Northumberland *Epipactis*. *Watsonia* **14**: 121–128.

ROBERTSON, A., NEWTON, A.C. & ENNOS, R.A. (2004). Multiple hybrid origins, genetic diversity and population genetic structure of two endemic *Sorbus* taxa on the Isle of Arran, Scotland. *Molecular Ecology* **13**: 123–134.

SQUIRRELL, J., HOLLINGSWORTH, P.M., BATEMAN, R.M., DICKSON, J.H., LIGHT, M.H.S., MACCONAILL, M. & TEBBITT, M.C. (2001). Partitioning and diversity of nuclear and

organelle markers in native and introduced populations of *Epipactis helleborine* (Orchidaceae). *American Journal of Botany* **88**: 1409–1418.

SQUIRRELL, J., HOLLINGSWORTH, P.M., BATEMAN, R.M., TEBBITT, M.C. & HOLLINGSWORTH, M.L. (2002). Taxonomic complexity and breeding system transitions: conservation genetics of the *Epipactis leptochila* complex (Orchidaceae). *Molecular Ecology* **11**: 1957–1964.

STACE, C.A. (1975). *Hybridization and the flora of the British Isles*. Academic Press, London.

STACE, C.A. (1989). *Plant taxonomy and biosystematics*, edn 3. Edward Arnold, London.

STACE, C.A. (1991). *New flora of the British Isles*, edn 1. Cambridge University Press, Cambridge.

STACE, C.A. (1997). *New flora of the British Isles*, edn 2. Cambridge University Press, Cambridge.

STACE, C.A., ELLIS, R.G., KENT, D.H. & MCCOSH, D.J. (eds) (2003). *Vice-county census catalogue of the vascular plants of Great Britain, the Isle of Man and the Channel Islands*. BSBI, London.

STEBBINS, G.L. (1957). Self fertilization and population variability in higher plants. *American Naturalist* **91**: 337–354.

STEBBINS, G.L. (1974). *Flowering plants: Evolution above the species level*. Belknap Press, Cambridge, Mass.

STEWART, A., PEARMAN, D.A. & PRESTON, C.D. (1994). *Scarce plants in Britain*. JNCC, Peterborough.

SUNDERMANN, H. (1970). *Europäische und Mediterrane orchideen: eine bestimmungsflora*. Brücke-Verlag Kurt Schmerson, Hannover.

U.K. BIODIVERSITY GROUP (1995). *Biodiversity: the UK steering group report*. HMSO, London.

WIGGINTON, M.J. (1999). *British red data books: 1 Vascular plants*. JNCC, Peterborough.

Current taxonomic research on the British & European flora
Bailey, J. & Ellis, R.G. (eds) 45–70 (2006), BSBI. London.

Molecular evolutionary rates shed new light on the relationships of *Festuca*, *Lolium*, *Vulpia* and related grasses (Loliinae, Pooideae, Poaceae)

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ABSTRACT

Festuca L., *Vulpia* Gmel. and *Lolium* L. are the three main genera of subtribe Loliinae, a group of temperate grasses characterized by their festucoid-type spikelet. Recent phylogenetic studies based on separate and combined analyses of nuclear ITS and chloroplast *trn*L-F nucleotide sequences have shown that the large genus *Festuca*, as traditionally circumscribed, is a paraphyletic assemblage of several Loliinae representatives. Monophyletic *Festuca* s.l. splits into two main diverging lineages, the 'broad-leaved *Festuca*' and the 'fine-leaved *Festuca*', each encompassing different genera and with several morphologically-intermediate taxa placed between them or at the base of the 'fine-leaved' clade. All these lineages include both perennial and annual taxa that show significant and parallel differences in rates of nucleotide substitution for the two genomes analyzed. Comparative rate analysis indicate that the 'fine-leaved *Festuca*' evolve, in general terms, faster than the 'broad-leaved *Festuca*', and have presumably been derived from them. Relative rate ratios support the minimum-generation-time hypothesis in both lineages, implying the existence of stabilized selection processes for the slow evolving perennials and rapid adaptive mechanisms for the rapidly evolving annuals. High divergences in rate heterogeneity may be responsible for unsatisfactory resolution at the basal node of the festucoid ITS tree. These results, together with conflicts between the ITS and the *trn*L-F trees in the placement of several taxa suggests that the existence of past reticulation events, might have profound implications on their classification.

Keywords: *Festuca*, Loliinae, *Lolium*, ITS, *trn* L-F, phylogeny, relative-rate-test, *Vulpia*.

INTRODUCTION

Festuca L. with *c.*500 species distributed in all five continents is the main genus of subtribe Loliinae, a group of temperate Pooid grasses characterized by their dorsally rounded lemma

and linear hilum. Taxonomic circumscription of *Festuca* and its close allies has changed over the past centuries. Clayton & Renvoize (1986) considered that *Lolium*, *Vulpia* and other small genera (*Castellia*, *Cynosurus*, *Lamarckia*, *Micropyropsis*, *Micropyrum*, *Psilurus*, and *Wangenheimia*, among others) were derived groups of *Festuca*, a proposal largely congruent with that of Tzvelev (1982) who restricted the festucoids to nine genera (*Bellardiochloa*, *Cutandia*, *Festuca*, *Loliolum*, *Lolium*, *Nardurus*, *Scleropoa*, *Sphenopus*, and *Vulpia*). *Festuca* has been historically divided into several subgenera and sections (Hackel 1882, 1887, 1906; Piper 1906; Krechetovich & Bobrov 1934; Krivotulenko 1960; Tzvelev 1971; and Alexeev 1977, 1978, 1980, 1981, 1986). Several segregates of this genus that were first included within it (*Vulpia*, *Schedonorus*, *Drymochloa*, and *Leucopoa*) have been recognized as independent genera at different times (Gmelin 1805; Cotton & Stace 1977; Stace 1981; Holub 1984, 1998; Tzvelev 1999, 2000; Soreng & Terrell 1998).

Lolium has been always treated as a genus different from *Festuca* based on its typical inflorescence traits related to its excavated inflorescence axis with sunken spikelets covered by a single glume. *Lolium* was first classified in its own subtribe Loliinae (Dumortier 1823) but later transferred to subtribe Festucineae C. Presl by Tzvelev (1982) based on morphology, karyology and hybridization traits. However, the name Loliinae Dumort. has nomenclatural priority over Festucineae C. Presl. (Soreng & Davis 2000). *Lolium* encompasses *c.*10-12 species that are mostly native to the Mediterranean region (Terrell 1968).

Vulpia was erected as a new genus by Gmelin (1805) and has usually been separated from *Festuca* ever since, based on on their annual habit, long unequal glumes, and long-awned lemma (Cotton & Stace 1977). The circumscription of *Vulpia* has changed depending on the inclusion or exclusion of different genera and infrageneric taxa (Stace 1981); up to five different sections have been recognized within *Vulpi*a based on breeding system and inflorescence traits (Cotton & Stace 1977; Stace 1987). The *Vulpia* taxa account for *c.*22 species native to the Mediterranean region and to America (Cotton & Stace 1976).

Lolium and *Vulpia* have been considered related to *Festuca* based on chromosome and breeding affinities (Jenkin 1933; Malik & Thomas 1966, Ainscough *et al.* 1986). *Lolium* hybridizes spontaneously with representatives of *Festuca* subgen. *Schedonorus* (a broad-leaved group) (Lewis 1975) and rarely with section *Aulaxyper* (a fine-leaved group), whereas *Vulpia* (sects. *Vulpia* and *Monachne*) intercrosses with those of *Festuca* sect. *Aulaxyper* (Barker & Stace 1982, 1984, 1986; Ainscough *et al.* 1986).

Other minor genera of the subtribe Loliinae have mostly been treated as independent genera more or less related to *Festuca* (Stace 1981, Clayton & Renvoize 1986). Up to eleven annual genera (*Castellia*, *Catapodium*, *Ctenopsis*, *Cutandia*, *Desmazeria*, *Loliolum*, *Micropyrum*, *Narduroides*, *Sclerochloa*, *Vulpiella*, *Wangenheimia*) were grouped in the *Vulpia-Desmazeria* complex by Stace (1981). Four of them (*Castellia*, *Ctenopsis*, *Micropyrum*, and *Wangenheimia*) were characterized as close allies of *Vulpia*, two others (*Loliolum* and *Narduroides*) as intermediate between *Vulpia* and *Desmazeria*, and the remainder as more related to *Desmazeria*. The short-perennial genus *Micropyropsis* was classified within Loliinae by Clayton & Renvize (1986).

A series of molecular phylogenetic studies on *Festuca* and its close satellites (Darbyshire & Warwick 1992; Charmet *et al.* 1997; Gaut *et al.* 2000; Torrecilla & Catalán 2002; Torrecilla *et al.* 2003, 2004; Catalán *et al.* 2004) demonstrated that *Festuca sensu lato* is a large paraphyletic assemblage that encompasses not only *Lolium* and *Vulpia* but also several other related genera. The most exhaustive survey of festucoid taxa conducted by Catalán *et al.* (2004) based on simultaneous analyses of nuclear ITS and chloroplast *trn*L-F sequences

found a likely evolutionary trend from more ancestral 'broad-leaved' *Festuca* lineages towards more recently derived 'fine-leaved' *Festuca* ones. In this study polyphyletic *Vulpia* and other Mediterranean ephemeral genera were nested within the 'fine-leaved' clade whereas *Lolium* and *Micropyropsis* were included within the 'broad-leaved' one. We also found the sister clades Dactylidinae and Cynosurinae/Parapholinae (in the sense of Soreng & Davis 2000) to be the closest relatives of Loliinae (cf. Catalán *et al.* 2004).

However, evolutionary rates within the Loliinae vary enormously showing a general trend from slow-evolving perennial lineages towards rapidly-evolving annual ones in the 'fine-leaved' clade (Torrecilla *et al.* 2004). An increasing number of investigations have demonstrated that most angiosperm groups exhibit strong differences in rates of nucleotide substitutions between closely related lineages in both nuclear and organellar genomes (Sanderson 1997; Muse 2000). The generation-time-effect hypothesis of the neutral theory connects differences in substitution rates with variable generation times; thus, organisms with shorter reproductive cycles exhibit higher molecular rates (Wu & Li 1985; Gaut *et al.* 1992).

Although this hypothesis has been widely utilized to explain the mechanisms involved in the evolution of sister groups in vertebrates, it has been contested in evolutionary studies of angiosperms due to the uncertainity of the number of germ line cells per replication (Gaut *et al.* 1992, 1997; Eyre-Walker & Gaut 1997). Because of this, Gaut *et al.* (1992) postulated an alternative hypothesis for the generation-time-effect in plants, the so-called minimum-generation-time (MGT) hypothesis, measured as the time from germination to first flowering.

Grasses have shown to be one of the most rapidly evolving lineages within monocotyledons and to respond in most cases, though not always, to the MGT hypothesis (Gaut *et al.* 1992, 1997). Different evolutionary trends in rate heterogeneity have been also associated with the speciation rate hypothesis which assumes that higher cladogenic events are derived from higher substitutional rates (Barraclough *et al.* 1996; Barraclough & Savolainen, 2001).

In our recent evolutionary study of a restricted group of Loliinae grasses (FEVRE: fine-leaved *Festuca* and related ephemerals; Torrecilla *et al.* 2004) we found a strong correlation between substitution rates in the ITS and *trn*L-F genome regions and the life-cycle strategies shown by these groups . Most annual FEVRE lineages exhibited significant higher mutational rates than their congeneric or cosectional perennial counterparts. Differences in rates of heterogeneity were found both within and between these annual and perennial 'fine-leaved' lineages. Further evidence suggested that other evolutionary processes, like hybridisation and polyploidy, could also have increased the evolutionary pace in some perennial and annual FEVRE groups (Torrecilla *et al.* 2004).

Because significant differences in nucleotide substitution rates within the 'fine-leaved' *Festuca* seem to be correlated with the generation-time-effect hypothesis (Torrecilla *et al.* 2004), we speculate that similar processes would be likely to have operated in the 'broad-leaved' *Festuca* where evolutionary trends towards short-life cycle or perennial polyploid vigour might have evolved independently in several lineages. Therefore, one of the aims of our present study is to test different evolutionary scenarios (i.e. MGT hypothesis, speciation hypothesis, reticulation/polyploidization hypothesis) that may be associated with different substitution rates across the festucoids based on the largest molecular analysis of this group of grasses conducted by Catalán *et al.* (2004). Because highly heterogeneous sequences may also be prone to accumulation of higher rates of homoplasy which could lead to undesirable long-branch attraction and site saturation effects in phylogenetic reconstruction (Bousquet *et*

al. 1992; Gaut *et al.* 1992, 1996, 1997), a second aim of our study is to estimate if highly heterogeneous rates could increase the risk of recovering potential artifactual relationships that may confound the classification proposals based on those phylogenies.

MATERIAL AND METHODS

PHYLOGENETIC ANALYSES

DNA sequence data of Loliinae representatives and other close subtribes is based on the phylogenetic survey of Catalán *et al.* (2004). A total sample of 119 representatives of subtribe Loliinae, 5 of subtribe Poinae (*Poa*, *Puccinellia*, *Sclerochloa*), 4 of subtribe Dactylidinae (*Dactylis*, *Lamarckia*), 2 of subtribe Cynosurinae (*Cynosurus*), 5 of subtribe Parapholinae (*Catapodium*, *Monerma*, *Parapholis*, *Sphenopus*), and one each of subtribes Sesleriinae (*Sesleria argentea*), and Psilurinae (*Psilurus incurvus*), together with 5 outgroup representatives of tribes Aveneae (*Avena barbata*, *A. eriantha* and *Deschampsia cespitosa*), Triticeae (*Secale cereale*) and Brachypodieae (*Brachypodium distachyon*) were used to conduct the phylogenetic analyses as indicated in Catalán *et al.* (2004). Within the festucoids (Loliinae), 71 samples corresponded to taxa of *Festuca* sensu lato, representing 5 subgenera and 12 sections, 18 corresponded to taxa of *Vulpia*, representing all the 5 sections of this genus, 10 corresponded to taxa of *Lolium*, and 9 corresponded to genera that have been considered or demonstrated to be more or less related to *Festuca* (*Castellia*, *Ctenopsis*, *Hellerochloa*, *Micropyropsis*, *Micropyrum*, *Narduroides*, *Parafestuca*, *Wangenheimia*). The list of taxa with authorities, localities, herbarium vouchers, ploidy levels and GenBank accessions is indicated in Catalán *et al.* (2004).

The ITS and *trn*L-F data matrices consisted of 117 and 111 sequences, respectively . The ITS data set was made of 644 aligned nucleotide characters of which 46% were informative, whereas the *trn*L-F data set was made of 1089 aligned nucleotide positions of which 21% were parsimony informative. Phylogenetic analyses were performed on each separate data matrix and on the combined data set as stated in Catalán *et al.* (2004). Bayesian inference approaches produced similar topologies to those obtained through parsimony based heuristic searches (Catalán *et al.* 2004). Bayesian trees allowed a preliminary estimation of the rates of evolution of the festucoid lineages based on their respective branch lengths. The Bayesian searches were performed using the program MRBAYES v. 3.0 (Huelsenbeck and Ronquist, 2002); the analysis of each separate data set was performed through 1,000,000 generations by the Markov Chain Monte Carlo (MCMC) sampling trees every 100 generations and allowing the program to estimate the respective likelihood parameters (nucleotide frequencies, nucleotide substitution rates, gamma shape, proportion of invariable sites). Sampled points from generations previous to stationarity were discarded using the burn-in option of Mr. Bayes 3.0. *Brachypodium distachyon* was used to root the trees. All trees sampled from these searches were used to construct the respective 50% majority-rule consensus trees where the percentage of times a clade is recovered is interpreted as an estimation of robustness.

RELATIVE RATE TESTS

Relative rate tests were performed between the main lineages of Loliinae and close allies for the two genome regions analyzed (ITS, *trn*L-F) in the search for significant differences in nucleotide substitution rates among them and as a means to test evolutionary models related

to: i) the minimum-generation-time (MGT) hypothesis, ii) the speciation rate hypothesis, and iii) the reticulation/polyploidization hypothesis within these grasses. The program RRTree (Robinson-Rechavi & Huchon 2000), which computes distance-based relative tests among pairs of groups of sequences (Robinson *et al.* 1998) based on a priori group covariances derived from the method of Li & Bousquet (1992), was used to conduct the tests. This program allows pairwise comparisons among groups taking into account a weighted topology or ignoring it.

Comparisons were performed for 31 groups of Loliinae and close allies that were circumscribed according to current classifications and to their respective phylogenetic resolution obtained in the ITS and *trn*L-F trees (Table 1). The Kimura two-parameter distance was used for these non-coding regions and topological references were based on the consensus maximum likelihood trees obtained from the respective ITS and *trn*L-F Bayesian analyses. As the sensitivity of the relative-rate test improves with higher taxonomic sampling (Robinson *et al.* 1998), the complete ITS and *trn*L-F data matrices were used to define the tested groups trimming in each case uncommon or incomplete sequences that could lead to anomalous results.

To improve the accuracy of the tests, appropriate outgroups for the Loliinae and close allies Dactylidinae and Parapholiinae/Cynosurinae were chosen from the close genus *Poa*. Perennial representatives with moderate rates of mutation were selected, respectively, for the ITS (*P. trivialis*) and *trn*L-F (*P. bulbosa*) tested analyses. Distance-based rate ratios were also computed among all pair groups without imposing topological weights; the observed differences were minimum (or none) indicating that the topological constraints do not seriously affect the conservative estimates of group covariances.

The MGT was tested according to the parameters established by Gaut *et al.* (1992, 1997). Annual festucoids flower within their single seasonal cycle (<1 yr) and have, therefore, shorter generation times than perennial festucoids, which usually flower after their first year (1-2 yr). Although some perennial species of *Festuca* exhibit clines for seedling establishment and longevity under different ecological conditions (Suzuki *et al.* 1999), patterns referred to when seed is first returned and for how many years seed is returned by an individual are practically unknown for most of the perennial fescues thus avoiding further perennial-class subdivision analysis in the present study.

The speciation rate hypothesis cannot be tested with confidence at the species level within the Loliinae groups as the specific and infraspecific hierarchical ranks and the numbers of the taxa attributed to some assemblages vary greatly depending on different authors. This hypothesis has therefore been tentatively assessed with respect to the number of genera that have been recognized within the main lineages of Loliinae (Holub, 1984, 1988; Watson & Dallwitz 1992) (Table 1).

We have developed here an estimate for the reticulation-and-polyploidization evolutionary scenario, predicted by previous authors for these highly hybridized groups of festucoid grasses (Ainscough *et al.* 1986; Soreng & Davis 2000), that is measured in terms of correlation between higher susbtitutional rates and higher ploidy levels (Table 1). Even if the nature of polyploidy across *Festuca* has long been debated (i.e. autopolyploidy vs. allopolyploidy), meiotic behaviour of artificial hybrids and molecular cytogenetic evidences support an hybrid allopolyploid origin for most of the broad- and fine-leaved *Festuca* complexes investigated so far (Jahuar 1975, 1993; Xu & Sleper 1994; Humphreys *et al.* 1995; Harper *et al.* 2004).

TABLE 1. BIOLOGICAL PARAMETERS FOR 31 GROUPS OF FESTUCOID GRASSES (SUBTRIBE LOLIINAE) AND ITS CLOSE ALLIES

Group	**Life-cycle[1]**	**MGT (yrs)[2]**	**Ploidy range**	**No. genera[3]**
Loliinae:				
Broad-leaved taxa:				5
Drymanthele	P	1-2	2x	
Leucopoa	P	1-2	6x-8x	
Subbulbosae	P	1-2	2x	
F. paniculata complex	P	1-2	2x-4x	
Scariosae	P	1-2	2x	
Pseudoscariosa	P	1-2	2x	
Schedonorus (European)	P	1-2	2x-6x	
Schedonorus (Maghrebian)	P	1-2	4x-10x	
Micropyropsis	P	1-2	-	
Lolium (perennial)	P	1-2	2x	
Lolium (annual)	A	<1	2x	
Subulatae	P	1-2	2x-4x	
Amphigenes	P	1-2	2x-4x	
F. californica	P	1-2	4x-8x	
Castellia	A	<1	-	
Fine-leaved taxa:				8
Eskia	P	1-2	2x (4x)	
Exaratae	P	1-2	2x (4x)	
Festuca	P	1-2	2x-6x	
Hellerochloa	P	1-2	-	
Aulaxyper	P	1-2	(2x) 6x-10x	
Micropyrum	A	<1	2x	
Wangenheimia	A	<1	2x	
Narduroides	A	<1	2x	
Ctenopsis	A	<1	2x	
Apalochloa	A	<1	2x	
Vulpia 2x	A	<1	2x	
Psilurus / *Vulpia* 4x-6x	A	<1	4x-6x	
Loretia complex	A	<1	2x (4x)	
Dactylidinae	A – P	<1 – 1-2	2x-4x	
Cynosurinae	A – P	<1 – 1-2	2x	
Parapholiinae complex	A	<1	2x-4x	

[1] A= annuals; P = perennials

[2] MGT = Minimum-generation-time (since germination to first flowering)

[3] Estimative values of the genera recognized by Holub (1984, 1988) and Watson and Dallwitz (1992) for the groups included in the present study.

RESULTS

THE ITS AND *TRNL-F* TREES

Bayesian trees obtained from the separate analyses of the ITS and *trn*L-F data matrices correspond to those indicated in Catalán *et al.* (2004). A test of goodness of fit for 56 nucleotide substitutions models that was previously conducted on each individual dataset using the likelihood ratio test statistic included in the program Model Test ver. 3.06 (Posada & Crandall 1998) showed the same optimal model (GTR+G+I, 4 gamma rate categories) for the two independent datasets.

The Bayesian search conducted on the ITS data set sampled 9931 trees which reached a stable likelihood value after the burn-in of 537 trees; the 50% majority rule consensus tree of all sampled trees is shown in Figure 1. The analysis of the *trn*L-F data set sampled 9681 trees, which reached a stable likelihood value after the burn-in of 300 trees; the 50% majority rule consensus tree of all sampled trees is shown in Figure 2. The two topologies are congruent in the resolution of a well to moderately supported clade of fine-leaved *Festuca* + *Vulpia* + Related Ephemerals (FEVRE group, cf. Torrecilla *et al.* 2004) in which the strongest support is for subclades *Aulaxyper* + *Vulpia* (2x), *Festuca*, and *Psilurus* / *Vulpia* (4x-6x). Representatives of *Eskia* and *Amphigenes* p.p. are resolved as basal paraphyletic assemblages of the FEVRE clade in both trees; *Wangenheimia* is resolved as the well supported sister taxon of the *Feštuca* clade in the *trn*L-F tree, whereas *Micropyrum* is unexpectedly resolved as sister to the *Aulaxyper* clade in the ITS tree (Figs 2 & 1, respectively). A fourth resolved but differently supported lineage is that of representatives of *Vulpia* sects. *Monachne* and *Loretia* plus *F. plicata*; the *trn*L-F tree also incorporates *Apalochloa* and *Ctenopsis* within it and shows an unresolved basal placement for representatives of *Festuca* subsect. *Exaratae* and close allies.

The broad-leaved group is resolved as monophyletic in the *trn*L-F tree (Fig. 2) whereas a series of broad-leaved lineages collapse with the FEVRE clade and with clades of other close subtribes at the basal node of the ITS tree (Fig. 1). The two topologies resolve a well-supported clade of *Lolium* + *Micropyropsis* + *Schedonorus*. Other resolved clades in the ITS tree (*Subbulbosae* pp, *F. paniculata* gr., *Leucopoa* , *Subulatae*, and *Scariosae* + *Pseudoscariosa* + *Drymanthele*) form a series of successive polytomies in the *trn*L-F tree. Some 'intermediate' taxa between the broad-leaved and the fine-leaved groups are nested in an odd position in one or the other tree (i.e *F. californica*) (Figs 1 & 2). *Castellia* is differently resolved in each topology whereas *Parafestuca* falls apart from the festucoid clade. *Lolium*, which is strongly resolved as monophyletic in the ITS tree, is shown to be paraphyletic in an intermingled clade of representatives of *Micropyropsis* and European *Schedonorus* in the *trn*L-F tree (Figs 1 & 2).

The combined analysis provides a better resolution than the separate analyses; Loliinae is resolved as monophyletic and separated into two diverging lineages, a well-supported clade of fine-leaved *Festuca* and a poorly supported clade of broad-leaved *Festuca* (Fig. 3). The presence of 'intermediate' taxa at the base of or close to the fine-leaved clade indicates a trend from more ancestral broad-leaved *Festuca* lineages towards the more recently evolved FEVRE lineages, a finding that is correlated with the high mutational rates observed in most of the annual lineages of the fine-leaved group (cf. Torrecilla *et al.* 2004). The combined analysis also resolved the sister clades Dactylidinae and Cynosurinae/Parapholinae as the closest relatives of subtribe Loliinae.

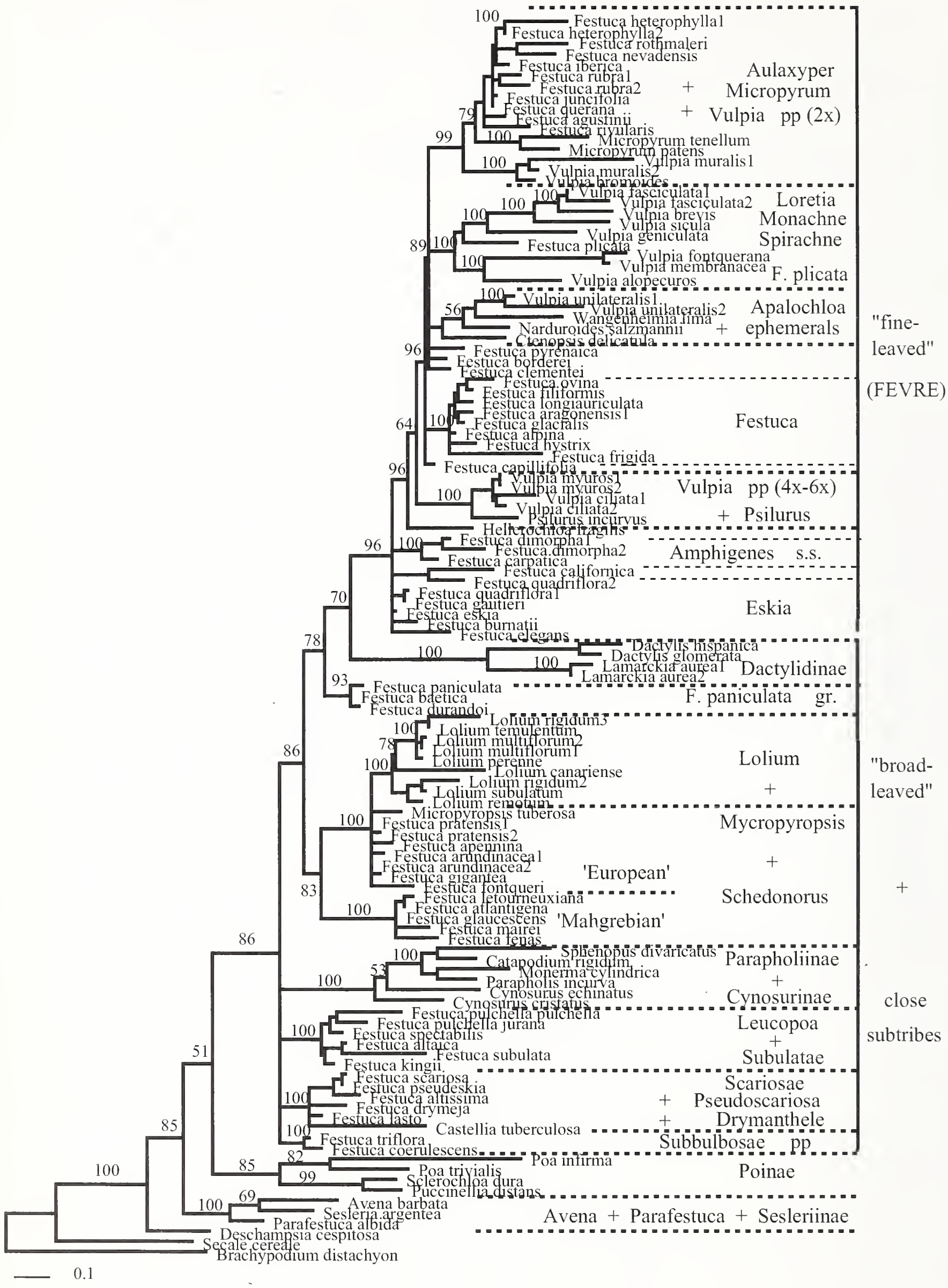

FIGURE 1. Bayesian ITS tree: 50% MR consensus tree of 9331 trees. Posterior probability percentages are indicated on the corresponding branches.

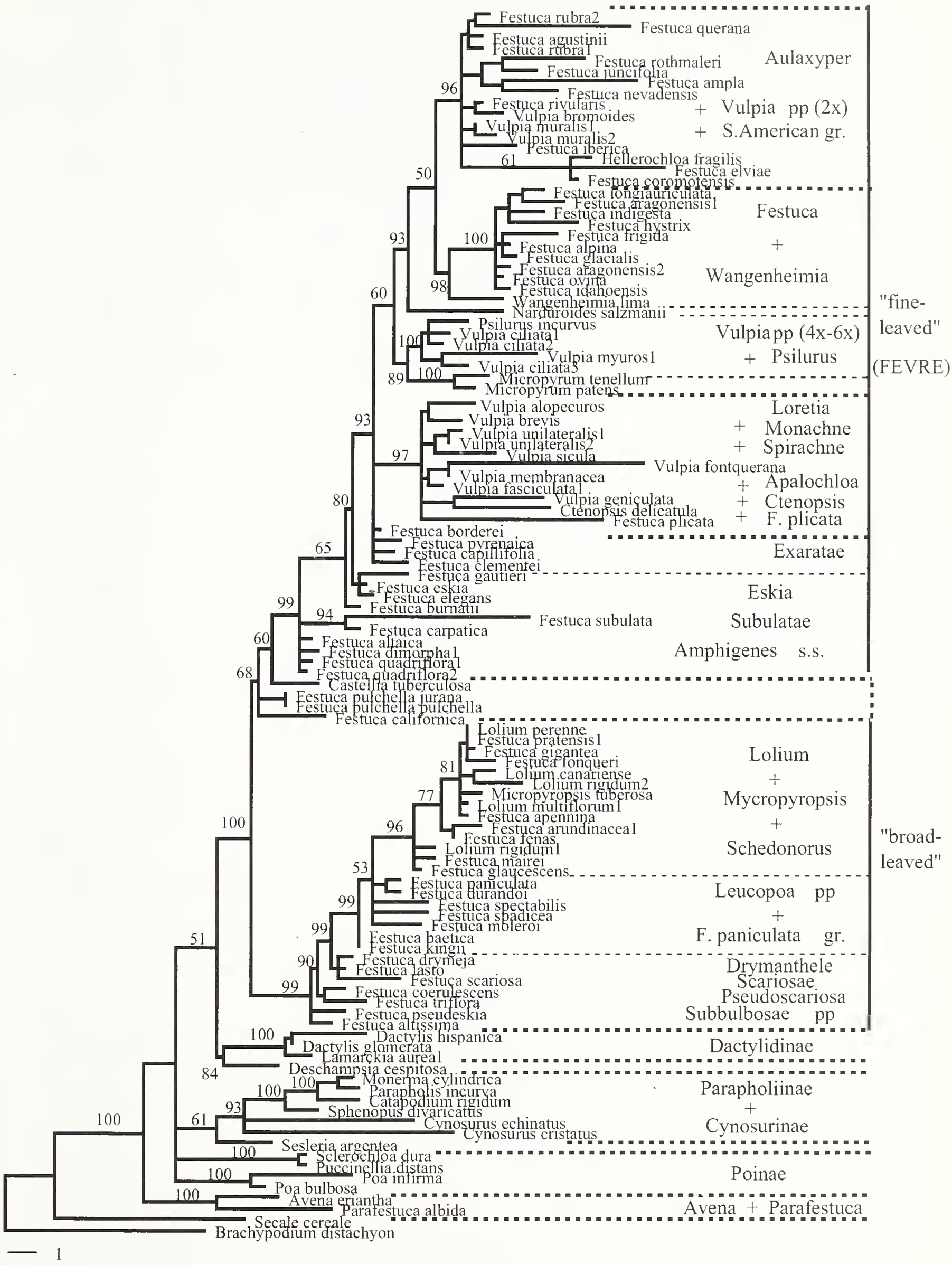

FIGURE 2. Bayesian *trn*L-F tree: 50% MR consensus tree of 9681 trees. Posterior probability percentages are indicated on the corresponding branches.

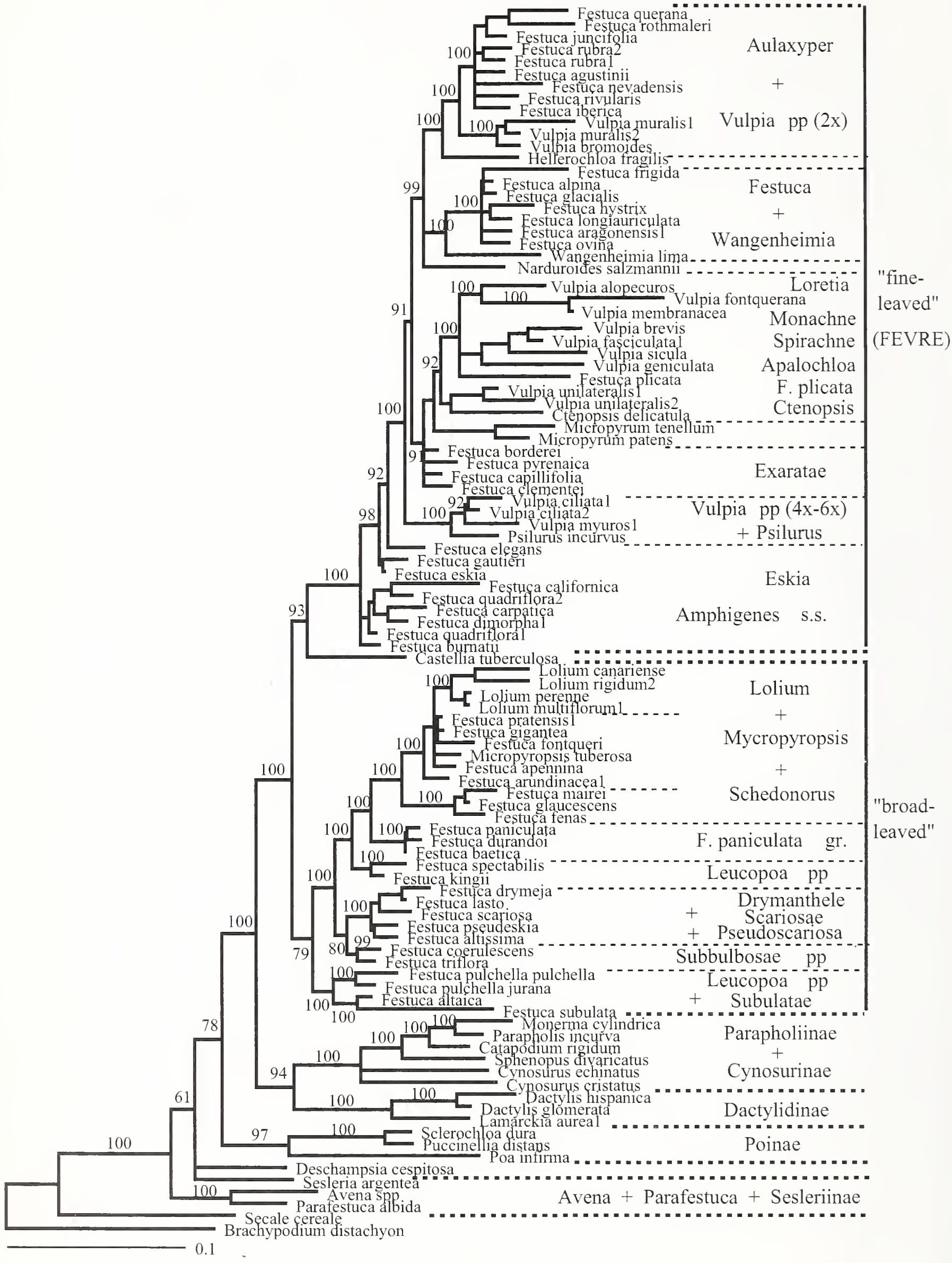

FIGURE 3. Bayesian combined ITS/*trn*L-F tree: 50% MR consensus tree of 6216 trees. Posterior probability percentages are indicated on the corresponding branches.

RELATIVE RATE TESTS

Of the 31 groups tested, three corresponded to the close subtribes Dactylidinae, Cynosurinae and Parapholiinae, and the remaining 28 to subtribe Loliinae (Table 1). Within this last lineage, 14 groups belong to the 'broad-leaved *Festuca*', another 13 to the 'fine-leaved *Festuca*', and 1 to the single unresolved taxon *Castellia*. The 'broad-leaved' lineage encompasses the groups *Amphigenes*, *Drymanthele*, *F. californica*, *F. paniculata* complex, *Leucopoa* (*F. kingii*, *F. spectabilis*), *Lolium*, *Micropyropsis*, *Pseudoscariosa*, *Scariosae*, *Schedonorus*, *Subbulbosae*, and *Subulatae*. *Schedonorus* was further subdivided into two subgroups, the 'European' *Schedonorus* and the 'Maghrebian' *Schedonorus*, based on their divergence in the ITS tree. *Lolium* was also halved into two subgroups, each of them including perennial/biannual (*L. perenne*, *L. multiflorum*) and annual (*L. rigidum*, *L. canariense*) species, respectively, for both ITS and *trn*L-F regions.

The 'fine-leaved' lineage presents a mixed array of perennial (*Aulaxyper*, *Eskia*, *Exaratae*, *Festuca*, *Hellerochloa*) and the mostly annual (*Apalochloa*, *Ctenopsis*, *Loretia* complex (*Spirachne*, *Monachne*, *Loretia*, *F. plicata*, *Micropyrum*, *Narduroides*, *Psilurus* complex (*Psilurus*, *Vulpia* 4x-6x), *Vulpia* (2x), *Wangenheimia*) groups.

Most of the chosen groups were monophyletic or corresponded to terminal branches; however, in a few cases some paraphyletic assemblages of closely related taxa were also taken into account. The minimum-generation-time (MGT) hypothesis, the speciation hypothesis, and the reticulation/polyploidization hypothesis were assessed for different groupings of festucoids. The results obtained from the conducted relative-rate tests are presented in Table 2 (A-F).

Compared to its closest relatives, Loliinae evolves slightly faster than Cynosuriinae and Parapholiinae but significantly slower than Dactylidinae for the ITS region (Table 2, E). The accelerated rate of mutation of the Dactylidinae ITS sequences is contrastingly higher than those presented by most assemblages of Loliinae and with respect to those of Cynosurinae and Parapholiinae (Table 2, A-D). Conversely, the more conserved substitutional rate of the chloroplast *trn*L-F region does not detect significant differences between the Loliinae and its closest relatives (Table 2, E) though levels of significance are manifested in more detailed comparisons among Loliinae groups and with respect to Cynosurinae (Table 2, A, B, D). Surprisingly, the Cynosurinae taxa show some of the fastest rates of *trn*L-F substitutions of all groups studied and are significantly higher than those of Dactylidinae, Parapholiinae, and the slowly-evolving Loliinae assemblages (Table 2, A, B).

Within Loliinae, the 'broad-leaved' groups evolve in general terms at a lower pace than the 'fine-leaved' ones for both genome regions (Table 2, E) though differences are more pronounced in the ITS region. The broad-leaved perennial assemblages of *Leucopoa*, *Drymanthele*, and *Subbulbosae*, which incude some of the tallest and most robust representatives of *Festuca*, show the lowest rates of substitution in all the Loliinae studied. Their rate differences are highly significant with respect to the rapidly evolving ephemeral groups of the fine-leaved *Festuca* clade (Table 2, A-D). Broad-leaved assemblages with slighly higher mutational rates are those of *Scariosae*, *Pseudoscariosa*, and the *F. paniculata* complex, which are close to those presented by the slow-evolving fine-leaved groups *Eskia* and *Exaratae* (Table 2, A-D). All these groups show significant differences from the fastest evolving ephemeral lineages of the fine-leaved clade.

The most rapidly evolving lineage within the broad-leaved clade is that of *Schedonorus*/*Micropyropsis*/*Lolium*. The 'European' *Schedonorus* evolve at a lower pace,

TABLE .2A. RELATIVE RATE RATIOS OBTAINED FROM RELATIVE-RATE-TESTS. THE RATIO IS GIVEN AS THE RATIO OF ROW OVER COLUMN. BOLD CARACTERS ARE SIGNIFICANT AT DIFFERENT P VALUES (* = P<0.05; ** = P<0.001; *** = P<0.0001).

ITS \ *trnL-F*	Dac	Cyn	Par	Dry	Leu	Subbul	Fpan	Sca	Pse	ScheE	ScheM	Microsi	Lolp	Lola	Subu	Amp	Fcal	Castellia
Dactylidinae	-	**-2.66****	0.12	0.29	-0.61	0.34	-0.98	-0.11	0.44	-1.60	-1.16	-1.44	-1.37	**-2.16***	**-3.9*****	-0.43	0.002	-0.22
Cynosuriinae	**-3.26****	-	**2.43***	**2.90****	**1.99****	**2.92****	1.46	**2.96****	**2.53***	1.04	1.30	0.79	1.09	0.09	**2.72****	**2.12***	**2.68****	**2.95****
Parapholiinae	**-3.20****	0.38	-	0.42	-0.47	0.46	-0.64	-0.63	0.55	-1.50	-0.94	-1.34	-1.27	-1.80	**-3.15****	-0.08	0.13	-0.07
Drymanthelé	**-4.6*****	-0.73	-1.43	-	-1.62	0.13	**-2.09***	-1.76	-0.11	**-2.88****	**-2.28***	**-2.26***	**-2.42***	**-2.92****	**-3.7*****	-0.85	-0.32	-0.55
Leucopoa	**-4.5*****	-1.01	-1.86	0.71	-	1.42	-0.71	-0.76	-1.11	-1.65	-1.06	-1.33	-1.37	**-2.18***	**-3.13****	0.23	0.58	0.33
Subbulbosae	**-4.7*****	-0.37	-1.30	-0.0005	-0.35	-	**-2.13***	-1.53	0.00003	**-2.73****	**-2.14***	**-2.25***	**-2.36***	**-3.14****	**-3.8*****	-0.93	-0.39	-0.62
F.paniculata	**-4.7*****	0.08	-1.29	-0.80	-0.19	0.10	-	1.40	1.36	-0.98	-0.63	-0.62	-0.55	-1.77	**-3.06****	0.49	1.24	1.49
Scariosae	**-4.4*****	-0.58	-1.32	-0.16	-0.40	-0.10	0.32	-	1.30	-0.89	0.13	-0.64	-0.59	**-2.52***	**-3.5*****	0.40	0.82	0.80
Pseudoscariosa	**-4.4*****	-0.57	-1.30	-0.13	-0.41	-0.10	0.17	0.00	-	**-2.35***	0.79	-1.93	**-2.00***	**-2.41***	**-3.7*****	-1.02	-0.50	-0.71
Schedonorus (E)	**-4.1*****	0.06	-0.94	0.68	0.94	0.47	0.39	0.32	0.31	-	1.11	0.31	1.17	**-2.09***	**-2.55***	1.34	1.74	1.49
Schedonorus(M)	**-2.94****	0.38	-0.11	1.27	1.74	1.13	1.22	0.99	0.99	0.97	-	-0.09	-0.35	-1.93	**-2.57***	0.92	1.21	0.92
Micropyropsis	**-3.4*****	0.43	-0.27	1.37	1.67	1.26	1.35	1.01	0.87	0.93	-0.47	-	0.28	**-2.37***	**-2.52***	1.15	1.49	1.28
Lolium(p)	**-3.5*****	-0.29	-0.42	1.30	1.46	0.96	0.87	0.77	0.77	0.88	-0.32	-0.03	-	**-2.61****	**-2.61****	1.09	1.47	1.25
Lolium(a)	-1.67	1.55	0.92	**2.89****	**3.14****	**2.71****	**2.53***	**2.16***	**2.17****	**3.61*****	**1.17**	**2.77****	**3.47*****	-	-1.82	1.73	**2.37***	**2.62****
Subulatae	-1.17	0.93	1.04	**2.17***	1.66	1.65	1.20	1.68	1.66	1.05	0.64	1.02	1.01	-1.41	-	**3.6*****	**3.9*****	**4.5*****
Amphigenes	**-1.96***	1.57	1.18	**2.54***	1.84	2.25*	1.80	**1.96***	**2.31***	1.60	0.59	1.07	0.97	-0.56	-0.14	-	0.49	0.28
F.californica	**-2.18***	**2.19***	0.91	**2.70****	1.83	**2.42***	**2.45***	**2.28***	**2.27***	1.91	1.13	1.43	1.38	1.12	0.47	0.99	-	-0.27
Castellia	**-2.93****	0.50	0.28	1.88	1.33	1.85	1.32	**2.08***	**2.09***	1.11	0.46	0.45	0.60	-0.70	-1.29	-0.61	0.87	-

Pairwise comparisons between Dactylidinae, Cynosurinae, Parapholiinae, and broad-leaved Loliinae groups (ITS: below diagonal; *trn*L-F: above diagonal). Abbreviations: Dac= Dactylidinae; Cyn=Cynosurinae; Par= Parapholiinae; Dry= *Drymanthele*; Leu= *Leucopoa*; Subbul= *Subbulbosae*; Fpan= *F.paniculata* complex; Sca= *Scariosae*; Pse= *Pseudoscariosa*; ScheE= 'European' *Schedonorus*; ScheM= 'Maghrebian'*Schedonorus*; Microsi= *Micropyropsis*; Lolium(p)= *Lolium* (perennials); Lolium(a)= *Lolium* (annuals); Subu= *Subbulatae*; Amp= *Amphigenes*; Fcal= *F.californica*; Cas=*Castellia*.

TABLE. 2B. RELATIVE RATE RATIOS OBTAINED FROM RELATIVE-RATE-TESTS. THE RATIO IS GIVEN AS THE RATIO OF ROW OVER COLUMN. BOLD CARACTERS ARE SIGNIFICANT AT DIFFERENT P VALUES (* = P<0.05; ** = P<0.001; *** = P<0.0001).

ITS \ *trnL-F*	Dac	Cyn	Para	Esk	Exa	Fest	Helle	Aulax	Micropum	Wan	Nar	Cten	Apal	Vul	Psi	Lor
Dactylidinae	-			0.04	-0.55	-1.78	-0.06	-1.36	-1.89	-1.65	**-2.25***	**-2.33***	-0.79	-1.46	**-2.16***	**-2.52***
Cynosuriinae		-		**2.64****	1.74	0.97	0.64	0.83	0.91	1.35	0.43	0.18	1.51	1.23	0.62	0.66
Parapholiinae			-	0.25	-0.41	-1.50	-0.37	-0.78	-1.53	-1.33	-1.95	**-1.96***	-0.66	-1.33	-1.50	-1.88
Eskia	**-4.4*****	-0.88	-0.64	-	-1.70	**-3.5*****	**-3.10****	**-3.3*****	-1.89	**2.66****	**-3.11****	**-4.3*****	**-2.44***	**2.83****	**-3.08****	**-5.5*****
Exaratae	**-3.7*****	-0.66	-0.22	**1.99***	-	**-2.29***	**-2.66****	**-2.11***	-0.75	1.04	**-2.24****	**-3.5*****	-1.38	1.64	-1.69	**-4.7*****
Festuca	**-3.4*****	-0.23	0.28	**2.9*****	1.60	-	-0.41	1.10	1.43	0.69	-0.07	-0.42	1.30	0.76	0.79	-0.61
Hellerochloa	**-2.5***	0.46	0.82	**2.18***	1.65	0.77	-	**2.22***	1.27	0.63	0.41	-0.37	1.87	1.59	1.36	-0.01
Aulaxyper	**-3.18****	0.07	0.75	**2.68****	1.68	0.61	-0.65	-	0.71	0.22	-0.07	-1.39	0.67	-0.57	0.09	-1.33
Micropyrum	-1.50	1.52	1.82	**3.8*****	**3.14****	**2.64****	**2.11***	0.43	-	-0.67	-1.48	**-2.57****	-0.14	-0.66	-0.95	**-2.84****
Wangenheimia	-1.71	1.56	1.85	**3.7*****	**2.97****	**2.30***	1.22	-0.89	-0.72	-	-0.43	-1.90	1.44	0.06	0.67	**-2.07***
Narduroides	-0.97	1.53	1.68	**2.80****	1.80	1.38	**2.05***	0.22	-1.34	-0.37	-	-1.18	-1.39	0.72	0.57	-1.17
Ctenopsis	**-2.73****	0.31	0.63	**3.8*****	**2.99****	1.74	-0.13	0.22	-0.51	-0.81	1.23	-	**2.83****	1.76	**2.14***	0.08
Apalochloa	**-3.00****	0.15	1.52	**4.1*****	**3.11****	1.47	-0.35	0.63	0.37	1.60	0.30	0.26	-	-0.47	-0.60	**-3.4*****
Vulpia 2x	-1.54	1.40	1.39	**3.7*****	**2.87****	1.42	1.06	0.21	-1.45	-1.00	-0.05	0.09	-0.98	-	0.06	1.65
Psilurus	**-3.00****	0.30	0.33	**3.4*****	**2.12***	1.72	-0.22	0.16	-1.32	-0.91	-0.24	-0.78	-0.18	-0.17	-	-1.79
Loretia	-0.61	**1.98***	**2.30***	**6.2*****	**5.6*****	**4.4*****	**2.48***	**2.14***	0.88	1.13	**2.23***	0.71	1.60	**2.07***	1.88	-

Pairwise comparisons between Dactylidinae, Cynosurinae, Parapholiinae, and fine-leaved Loliinae groups (ITS: below diagonal; *trn*L-F: above diagonal). Abbreviations: Dac= Dactylidinae; Cyn= Cynosurinae; Par= Parapholiinae; Esk= *Eskia*; Exa= *Exaratae*; Fest= *Festuca*; Helle= *Hellerochloa*; Aulax= *Aulaxyper*; Micropum= *Micropyrum*; Wan= *Wangenheimia*; Nar= *Narduroides*; Cte= *Ctenopsis*; Apal= *Apalochloa*; Vul= *Vulpia* (2x); Psi= *Psilurus* complex (*Psilurus*//*Vulpia*4x-6x); Lor= *Loretia* complex.

TABLE. 2C. RELATIVE RATE RATIOS OBTAINED FROM RELATIVE-RATE-TESTS. THE RATIO IS GIVEN AS THE RATIO OF ROW OVER COLUMN. BOLD CARACTERS ARE SIGNIFICANT AT DIFFERENT P VALUES (* = P<0.05; ** = P<0.001; *** = P<0.0001).

ITS	Eskia	Exa	Fest	Helle	Aulax	Micropum	Wan	Nar	Cten	Apal	Vul	Psi	Lor
Drymanthele	-1.22	-1.25	-1.93	**-2.27***	**-2.07***	**-2.60****	**-3.4*****	**-2.45***	**-2.10***	**-2.14***	**-3.19****	**-2.24***	**-3.9*****
Leucopoa	-0.37	-0.77	-0.97	-1.70	-1.41	**-2.04***	**-2.88****	-1.83	-1.75	-1.39	**-2.52***	-1.57	**-3.04****
Subbulbosae	-1.04	-1.17	-1.50	**-2.17***	-1.50	**-2.31***	**-3.05****	**-2.10***	-1.83	-1.74	**-2.77****	-1.85	**-3.5*****
F.paniculata	-1.20	-0.98	-1.47	-1.94	-1.62	-1.92	**-3.08****	-1.61	-1.77	-1.69	**-2.72****	-1.70	**-3.6*****
Scariosae	-0.84	-0.94	-1.41	-1.87	-1.54	**-2.40***	**-3.00****	**-2.06***	-1.66	**-2.74****	**-2.57***	-1.67	**-3.4*****
Pseudoscariosa	-0.76	-0.96	-1.78	-1.87	-1.42	**-2.36***	**-3.00****	**-2.05***	-1.65	**-2.74****	**-2.35***	-1.42	**-3.5*****
Schedonorus(E)	-0.32	-0.66	-0.87	-1.58	-1.83	**-2.09***	**-2.49***	-1.53	-1.34	-1.28	**-2.36***	-1.29	**-3.07****
Schedonorus(M)	0.83	0.35	-0.05	-1.05	-1.20	-1.79	**-1.98***	-1.30	-0.61	-0.66	-1.65	-0.65	**-2.14***
Micropyropsis	0.02	-0.23	-0.78	-1.16	-0.67	-1.53	**-1.99***	-1.24	-0.72	-1.73	-1.75	-0.43	**-2.89****
Lolium(p)	0.21	-0.27	-0.33	-0.97	-1.10	-1.61	-1.80	-1.21	-0.83	-0.58	-1.76	-0.72	**-2.35***
Lolium(a)	1.68	1.21	0.98	0.12	0.003	-0.48	-0.64	-0.13	0.40	0.64	-0.50	0.53	-1.05
Subulatae	1.29	0.80	0.43	0.91	0.73	-0.15	-0.61	-0.18	-0.32	-0.02	-0.24	0.21	-1.38
Castellia	0.48	0.42	0.13	-0.41	0.20	-1.20	-1.46	-1.04	-0.28	-0.006	-1.14	-0.14	**-2.11***
Amphigenes	**2.35***	1.32	0.64	0.68	0.89	-0.92	-1.84	-0.59	-0.39	-0.002	-0.80	-0.06	**-2.46***
F.californica	**2.45***	1.59	1.26	0.52	1.13	-0.13	-0.72	0.53	0.60	0.85	-0.54	0.72	-1.04

Pairwise comparisons between broad-leaved and fine-leaved Loliinae groups (ITS). Abbreviations as indicated in A) and B).

TABLE. 2D. RELATIVE RATE RATIOS OBTAINED FROM RELATIVE-RATE-TESTS. THE RATIO IS GIVEN AS THE RATIO OF ROW OVER COLUMN. BOLD CARACTERS ARE SIGNIFICANT AT DIFFERENT P VALUES (* = P<0.05; ** = P<0.001; *** = P<0.0001).

trnL-F	Esk	Exa	Fest	Helle	Aulax	Micropum	Wan	Nar	Cten	Apal	Vul	Psi	Lor
Drymanthele	-0.33	-1.00	**-2.26***	**-2.71****	-1.75	**-2.37***	**-2.46***	**-2.64****	**-2.75****	-1.21	**-2.24***	**-2.37***	**-3.12****
Leucopoa	0.79	-0.03	-1.34	-1.87	-0.85	-1.39	-1.49	-1.63	**-2.05***	-0.31	-1.17	-1.44	**-2.56***
Subbulbosae	-0.39	-1.06	**-2.34***	**-2.95****	**-1.99***	**-2.39***	**-2.53***	**-2.68****	**-2.68****	-1.28	**-2.45***	**-2.62****	**-3.21****
F.paniculata	0.86	0.30	-1.37	-1.16	-0.47	-1.01	-1.35	-1.35	-1.89	-0.21	-1.03	-0.90	**-2.04***
Scariosae	0.93	0.27	-0.96	**-2.39***	-0.56	-0.79	-1.21	-1.34	-1.59	-0.0007	-0.87	-0.84	**-2.00***
Pseudoscariosa	-0.53	-1.18	**-2.54***	**-2.82****	-1.87	**-2.20***	**-2.61****	**-2.75****	**-2.84****	-1.37	**-2.41***	**-2.59****	**-3.24****
Schedonorus(E)	1.93	1.19	-0.13	-0.90	0.18	-0.07	-0.42	-0.73	-0.85	0.87	-0.02	-0.17	-0.91
Schedonorus(M)	1.36	0.66	-0.50	-1.35	0.11	-0.71	-0.77	-1.08	-1.39	0.35	-0.29	-0.42	-1.53
Micropyropsis	1.61	0.98	-0.41	-1.17	0.25	-0.18	-0.34	-0.82	-0.92	0.71	-0.13	-0.05	-0.92
Lolium(p)	1.63	0.95	-0.33	-1.31	-0.05	-0.28	-0.61	-0.91	-1.02	0.65	-0.23	-0.35	-1.05
Lolium(a)	2.09	1.49	0.03	-0.87	0.66	0.39	-0.08	-0.08	-0.71	1.02	0.42	0.20	-0.92
Subulatae	**4.0*****	**3.5*****	**2.18***	**3.15****	**3.4*****	**2.84****	**2.29***	1.89	1.04	**2.91****	**2.89****	**3.20****	**1.97***
Castellia	0.36	-0.73	-1.63	**-3.28****	-1.52	**-2.07***	-1.88	**-2.28***	**-2.47***	-0.74	-1.55	**-2.08***	**-2.77****
Amphigenes	1.08	-0.23	**-1.98***	**-2.54***	-1.66	-1.85	-1.92	**-2.39***	**-2.55***	-0.62	-1.59	**-2.09***	**-3.16****
F.californica	0.04	-0.62	-1.68	**-2.56***	**-1.98***	**-2.10***	-1.78	**-2.31***	**-2.35***	-0.89	-1.62	**-2.11***	**-2.55***

Pairwise comparisons between broad-leaved and fine-leaved Loliinae groups (*trn*L-F). Abbreviations as indicated in A) and B).

TABLE. 2E. RELATIVE RATE RATIOS OBTAINED FROM RELATIVE-RATE-TESTS. THE RATIO IS GIVEN AS THE RATIO OF ROW OVER COLUMN. BOLD CARACTERS ARE SIGNIFICANT AT DIFFERENT P VALUES (* = P<0.05; ** = P<0.001; *** = P<0.0001).

Group1	Group2	ITS		*trn*L-F	
		ratio	probability	ratio	probability
Loliinae	Dactylidinae	-2.2121	**0.0269***	-0.0934	0.9295
Loliinae	Cynosuriinae	0.5950	0.5517	-0.7449	0.4563
Loliinae	Parapholiinae	0.7614	0.4464	0.0029	0.9976

Pairwise comparisons between Loliinae and related allies.

TABLE. 2F. RELATIVE RATE RATIOS OBTAINED FROM RELATIVE-RATE-TESTS. THE RATIO IS GIVEN AS THE RATIO OF ROW OVER COLUMN. BOLD CARACTERS ARE SIGNIFICANT AT DIFFERENT P VALUES (* = P<0.05; ** = P<0.001; *** = P<0.0001).

Group1	Group2	ITS		*trn*L-F	
		ratio	probability	ratio	probability
(1):					
Loliinae(a)	Loliinae(p)	3.9213	**0.00008*****	2.2048	**0.0274***
Broad-leaved(a)	Broad-leaved(p)	2.6869	**0.0072****	2.6945	**0.0070****
Fine-leaved(a)	Fine-leaved(p)	3.1219	**0.0018****	0.9565	0.3388
Dactylidinae(a)	Dactylidinae(p)	0.3478	0.7279	-0.7302	0.4652
Cynosurinae(a)	Cynosurinae(p)	1.7565	0.0790	-1.9320	0.0533
Parapholiinae(a)	Cynosurinae(p)	1.0432	0.2968	-2.7209	**0.0065****
(2):					
Fine-leaved	Broad-leaved	1.894	0.0582	0.6091	0.5424
(3):					
Loliinae(2x)	Loliinae(>2x)	-0.1493	0.8813	-2.3280	**0.0193***
Loliinae(p)(2x)	Loliinae(p)(>2x)	-0.1658	0.0971	-3.0005	**0.0027****
Loliinae(a)(2x)	Loliinae(a)(>2x)	2.3739	**0.0176***	0.5959	0.5511
Broad-leaved(2x)	Broad-leaved(>2x)	0.1515	0.8795	-0.5496	0.5825
Broad-leaved(p)(2x)	Broad-leaved(p)(>2x)	-0.6807	0.4960	-0.6793	0.4968
Fine-leaved(2x)	Fine-leaved(>2x)	0.0788	0.9371	-2.4615	**0.0138***
Fine-leaved(p)(2x)	Fine-leaved(p)(>2x)	-1.1162	0.2643	-3.1404	**0.0017****
Fine-leaved(a)(2x)	Fine-leaved(a)(>2x)	2.4504	0.0143*	2.1081	**0.0350***
Dactylidinae(2x)	Dactylidinae(>2x)	-1.5016	0.1332	3.2813	**0.00103****
Cynosurinae(2x)	Parapholiinae(4x)	0.2275	0.8199	1.3381	0.1808

Relative-rate-tests ratios for the Minimum Generation Time (MGT) hypothesis (1), the speciation-rate hypothesis (2), and the reticulation/polyploidization hypothesis (3). Abbreviations: (a) = annuals; (p) = perennials; (2x) = diploids; (>2x) = polyploids.

followed by *Micropyropsis* and the 'Maghrebian' *Schedonorus* group, and then by *Lolium*. Further differences within the last group indicate that the perennial/biennial *Lolium* taxa evolve at a rate similar to or slightly lower than *Micropyropsis*, whereas the annual *Lolium* taxa show accelerated substitution rates at both ITS and *trn*L-F regions, being significantly different from most of the remaining broad-leaved groups and as fast (or faster) than some of the fine-leaved groups (i.e. *Apalochloa*, *Aulaxyper*, *Festuca*, *Hellerochloa*, *Psilurus*) (Table 2, A, C, D).

Groups of 'intermediate' placement (i.e. *Amphigenes*, *Castellia, F. californica*) in the combined Bayesian tree also show intermediate rates of mutation between the slow-evolving ones of the 'broad-leaved' groups and the fast-evolving ones of the 'fine-leaved' groups (Table 2, A, C, D).

The more advanced 'fine-leaved' *Festuca* groups show a trend towards higher substitutional rates in both ITS and *trn*L-F sequences. Rate heterogeneity ranges from the lowest ones corresponding to old relict perennial groups (*Eskia*, *Exaratae*), through the intermediate ones of more recently evolved perennials (*Aulaxyper*, *Festuca*, *Hellerochloa*), to the fastest ones acquired by the newly derived annuals lineages. Within the last group different assemblages of the genus *Vulpia* and its close allies show significant differences from most of the remaining 'fine-leaved' groups for the ITS (*Loretia*) and the *trn*L-F (*Loretia*, *Ctenopsis*) sequences (Table 2, B-D).

Relative rate tests designed to test the MGT hypothesis indicate that annual lineages evolve faster than the perennial ones within the studied groups of Poeae grasses, though differences are only significant within the more largely sampled Loliinae lineage at both ITS and *trn*L-F regions (Table 2, F). Broad-leaved annual taxa (*Lolium* spp.) show significant differences in ITS and *trn*L-F sequences from the rest of the broad-leaved groups whereas fine-leaved annual taxa (*Vulpia* and other ephemerals) are significantly different from other fine-leaved groups only in ITS sequences. The close subtribes Dactylidinae and Cynosurinae/Parapholiinae also show higher but non-significant mutational rates in annuals than in perennials for the ITS sequences; however, the reverse pattern is observed for the chloroplast *trn*L-F sequences where perennials show higher (but non-significant) rates than the annuals. The limited taxon sampling of these last groups avoids further speculation on the lack of correlation detected for the *trn*L-F data set with respect to the MGT hypothesis.

The speciation rate hypothesis was only estimated between the 'fine-leaved' and the 'broad-leaved' lineages of *Festuca* based on the recognized genera included in our present survey (Table 2, F). The more diverse 'fine-leaved' *Festuca* evolve faster than the less diverse 'broad-leaved' ones, though those differences were not significant, indicating a relative support for the cladogenetic process.

A new evolutionary scenario has been confirmed for the perennial groups of Loliinae with respect to the reticulation/polyploidization hypothesis (Table 2, F). Highly polyploid taxa show higher substitutional rates than their respective ingroup diploid counterparts at both ITS and *trn*L-F regions, though those differences are not always significant. Conversely, diploid annual taxa tend to show higher mutational rates than the ephemeral polyploids (Table 2, F). Although hybridization may have affected equally diploid and polyploid lineages, it is a more common phenomenon within the latter groups, which sorted out the new sterility barriers via recurrent introgression and polyploidization (Stebbins 1956; Stace 1987). Polyploidization is expected to increase the rate of variability of the nuclear genome concordantly with the accumulation of more gene copies (Soltis & Soltis 1999), but should leave the chloroplast genome less affected. However, concurrent rates of nucleotide substi-

tutions in the two genomes, detected for either the diploid and the polyploid lineages of the Loliinae, may be indicative of other concerted nuclear and cytoplasm replication mechanisms that could be operating in these plant cells (Gaut *et al.* 1997).

DISCUSSION

HETEROGENEITY RATES AND THE PHYLOGENY OF THE FESTUCOIDS (SUBTRIBE LOLIINAE)

A more comprehensive phylogenetic framework for *Festuca s.l.* was drawn after the molecular survey of Catalán *et al.* (2004) and the present study. It has been demonstrated that the paraphyletic *Festuca* lineage encompasses not only *Lolium* and *Vulpia*, but also other genera that are nested either within the 'fine-leaved' *Festuca* (*Ctenopsis*, *Hellerochloa*, *Micropyrum*, *Narduroides*, *Psilurus*, *Wangenheimia*), within the 'broad-leaved' *Festuca* (*Micropyropsis*), or in an 'intermediate' position between them (*Castellia*). The festucoid lineage emerges as a natural group. Our studies have also shown that subtribe Loliineae and its close relatives Dactylidinae and Cynosurinae/Parapholiinae form a monophyletic and well supported group within the Poeae (Fig. 3).

Relative rate tests have demonstrated that the Dactylidinae taxa show extremely accelerated substitution rates in ITS sequences compared to other analyzed groups indicating a release from stabilized selection (Bousquet *et al.* 1992; Muse, 2000). High mutational rates may have negative consequences on phylogenetic reconstructions due to the loss of deep phylogenetic signal which conveys undesirable results like the long-branching attraction and site saturation effects (Wendel & Doyle 1998; Hillis & Wiens 2000). The lack of resolution observed at the basal node of the festucoid ITS Bayesian tree (Fig. 1) and the unexpected sister relationship of Dactylidinae to the fine-leaved clade can be associated with disturbing effects caused by increasing levels of homoplasy displayed by this rapidly evolving group. Potential arctifactual placements of *Dactylis* with respect to the fine-leaved *Festuca* were also observed in the phylogenetic trees of Charmet *et al.* (1997) and Lehväslaiho *et al.* (1987); however, our chloroplast *trn*L-F data does not indicate an accelerated rate of nucleotide substitution for the Dactylidinae, and the relationships recovered here indicate that Dactylidinae is a close relative but not part of the Loliinae assemblage.

Relative rate ratios show that the Cynosurinae/Parapholinae ITS sequences evolve at an intermediate pace between the slow and fast evolving Loliinae lineages, though the Cynosurinae taxa show significant differences in rate substitution for the chloroplast *trn*L-F region with respect to most of the groups studied. These differences, however, do not seem to have altered the topology of the Loliinae clade neither the basal position of Cynosurinae with respect to the Parapholiinae group, which is congruent with the resolution obtained from the ITS data.

THE 'BROAD-LEAVED' *FESTUCA*, *MICROPYROPSIS* AND *LOLIUM*

The typical dichotomy between 'broad-leaved' *Festuca* and 'fine-leaved' *Festuca* lineages observed in previous studies of *Festuca* based on more limited taxon sampling (Torrecilla & Catalán 2002) became blurred when sampling of festucoid representatives was increased (Catalán *et al.* 2004). The presence of a series of unresolved clades and polytomies in the ITS and *trn*L-F based trees, together with the doubtful position of several 'intermediate' taxa

between the two lineages, suggests a basal paraphyly of the 'broad-leaved' lineages of *Festuca* s. l. with respect to the more recently evolved 'fine-leaved' ones. However, a poorly supported clade of broad-leaved taxa is still recovered in the combined ITS/*trn*L-F analyses (Fig. 3). *Lolium* is nested within a paraphyletic *Schedonorus* clade; evidence from the two separate analyses (Figs 1 & 2) suggests that *Lolium* is of recent origin and probably evolved from a European *Schedonorus* ancestor.

Nucleotide substitution rates parallel the evolutionary relationships recovered in the phylogenetic trees for the broad-leaved *Festuca* lineages. The slowest rates shown by *Drymanthele*, *Subbulbosae* and *Leucopoa* s.s. reflect their basal positions in the Loliinae trees, whereas the 'intermediately' placed *Amphigenes*, *Breviaristatae* (*F. californica*) and *Subulatae* (*F. subulata*) also show overall intermediate rates of nucleotide mutation. The *trn*L-F sequence of *F. subulata* constitutes a singular case as this taxon presents significant differences in chloroplast rate mutation with respect to all remaining lineages except Cynosurinae. The accelerated rate of mutation shown by *F. subulata* could also have affected its phylogenetic placement in the *trn*L-F trees due to long-branch attraction or site saturation effects.

Relative rate ratios corroborate the recent origin of the *Schedonorus /Micropyropsis/Lolium* lineage within the broad-leaved *Festuca* clade. Our analyses indicate that this group has the fastest mutational rates for both ITS and *trn*L-F sequences in a trend ranging from slow-evolving 'European' *Schedonorus* through intermediate-evolving *Micropyropsis* and 'Maghrebian' *Schedonorus* towards rapidly-evolving *Lolium*. 'Maghrebian' *Schedonorus* forms a clade of highly polyploid taxa presumably derived from more ancestral diploid and low polyploid 'European' *Schedonorus* ancestors (Borrill *et al.* 1977). Within *Lolium,* the annual taxa show highly accelerated mutational rates significantly different from most of the slow-evolving perennial lineages. The high levels of morphological variability detected in *L. rigidum* and *L. canariensis*, which moved some authors to describe different infraspecific and specific taxa out of those complexes (Terrell 1968; Scholz *et al.* 2000), are thus correlated with their higher susbtitutional rates.

THE 'FINE-LEAVED' *FESTUCA*, *VULPIA* AND RELATED EPHEMERALS

The 'fine-leaved' *Festuca* are resolved as monophyletic in all separate and combined analyses (Figs 1-3). Resolution of the main groups correspond to that described by Torrecilla *et al.* (2004) and Catalán *et al.* (2004) for the *Festuca* + *Vulpia* + Related Ephemerals (FEVRE) group with the addition of a group of S. American taxa (*Festuca coromotensis*, *F. elviae, Hellerochloa fragilis*) that belong to the clade of red fescues (*Festuca* sect. *Aulaxyper*) in the *trn*L-F based tree (Fig. 2). The four best supported clades correspond to those of the *Festuca* (+ *Wangenheimia*) clade, the *Aulaxyper* + *Vulpia* p.p. (2x) clade, the *Psilurus* + *Vulpia* p.p. (4x-6x) clade, and the *Loretia-Monachne-Spirachne* + *Festuca plicata* clade (Figs 1-3).

Surprisingly, *Vulpia* is resolved as polyphyletic in both nuclear and chloroplast trees. This striking finding especially affects the divergent and robust resolution obtained for representatives of typical section *Vulpia*: a clade of diploid taxa which is closely related to the *Aulaxyper* group whereas higher ploidy-level taxa link with *Psilurus* in an unrelated polyploid clade (Figs 1 & 2).

Relative rates of nucleotide substitutions for the 'fine-leaved' *Festuca* groups also correlate well with their recovered phylogenetic relationships. The basal *Eskia* and *Exaratae*

assemblages are shown to be the slowest-evolving groups within the clade followed by the intermediate-evolving *Festuca*, *Aulaxyper* and *Hellerochloa* lineages, and then by the rapidly evolving annual groups. Changes in rate heterogeneity vary greatly within the perennial fine-leaved groups, being more pronounced in the highly heterogeneous *Aulaxyper* group. Within this lineage, the high polyploid taxa (8x-10x) show the highest subtitutional rates in their ITS sequences (Results not shown).

The ephemeral fine-leaved groups are the most rapidly evolving festucoids as indicated by their relative rate ratios; rate differences are highly significant for the *Loretia* assemblage. Homoplasy may be enhanced in these highly evolving lineages altering, therefore, the phylogenetic inference. However, the consistent *Loretia /Monachne/ Spirachne /Apalochloa +F. plicata* clade in both ITS and *trn*L-F analyses support a common ancestor for all *Vulpia* lineages (the '*Loretia* assemblage') except for typical sect. *Vulpia*. The odd position of diploid and polyploid taxa of sect. *Vulpia* in the ITS and *trn*L-F trees cannot be explained in terms of significant differences in rate heterogeneity between them (Table 2 B).

MINIMUM-GENERATION-TIME AND RETICULATION/POLYPLOIDIZATION HYPOTHESES ARE THE LIKELY EVOLUTIONAY SCENARIOS FOR THE FESTUCOIDS

The relative rate analyses designed to test the MGT hypothesis within the festucoids and their closest allies have demonstrated that there are significant differences between molecular evolutionary rates of annual and perennial groups in the broadly sampled Loliinae. Thus, we can conclude that MGT mechanisms are operating in the rapidly evolving ephemeral groups of these grass lineages even if the biological factors that regulate these processes have not yet been deciphered (Gaut *et al.* 1997; Muse 2000). Changes in the evolutionary rates between slowly-evolving perennials and fast-evolving annuals are likely to be a consequence of a release in stabilized selection followed by the ephemeral groups which also facilitated the acquisition by them of rapid adaptive changes to new environmental habitats (Givnish 1997). Our study suggests that this evolutionary scenario has occurred in parallel along the two main lineages of broad-leaved and fine-leaved *Festuca*. Most interestingly, the substitutional rates of the annual representatives of the broad-leaved clade (*Lolium*) are similar to some substitutional rates of annual representatives of the fine-leaved clade (*Apalochloa, Psilurus*/*Vulpia* 4x-6x, *Vulpia* 2x) although most of the fine-leaved ephemerals evolve at a higher pace (*Loretia* assemblage, *Wangenheimia*). The extended presence of annual lineages derived from the fine-leaved fescues and their higher evolutionary rates are indicative of their more recent origin.

Transilience is also manifested along the perennial groups that show a shift from low to high rates of mutation. The increased evolutionary rates are correlated with increased levels of ploidy supporting the reticulation/polyploidization hypothesis tested here. Again, this evolutionary scenario has been developed independently along the two main clades of Loliinae, as exemplified by the *Aulaxyper* and the 'Maghrebian' *Schedonorus* groups within the 'fine-leaved' and 'broad-leaved' *Festuca* lineages, respectively. These two groups encompass highly polyploid taxa (8x-10x) that are presumably derived from their respective lower-ploidy-level relatives. Diploid perennial lineages display the lowest mutational rates in both 'broad-leaved' and 'fine-leaved' *Festuca* lineages, though the broad-leaved ones evolve significantly more slowly. Thus, according to previous evolutionary predictions by

Hackel (1882) and Tzvelez (1971), the nemoral and broad-leaved taxa of subgen. *Drymanthele* and sect. *Subbulbosae* probably constitute some of the oldest relict lineages of *Festuca*.

The minimum-generation-time hypothesis was not significantly supported in the close subtribes Dactylidinae and Cynosurinae/Parapholiinae, though it might be caused by the lower taxon sampling within these groups or by other phenomena related to hybridisation and polyploidy that might have fostered the acquisition of the overall accelerated rates shown by these taxa. In general terms, our results agree with the conclusions drawn from the study of Gaut *et al.* (1997) who found relative support for the MGT hypothesis within the grasses and with respect to the fastest evolving subfamily Pooideae.

Preliminary analyses also favour the speciation rate hypothesis within Loliinae, as indicated by the higher diversifying rates shown by the highly accelerated fine-leaved Loliinae lineages compared to the less-diversified rates of the slowly mutational broad-leaved ones. Diversification has been measured here with respect to the number of genera traditionally recognized within the 'fine-leaved' clade (*Ctenopsis*, *Festuca* p.p., *Hellerochloa*, *Micropyrum*, *Narduroides*, *Psilurus*, *Vulpia* p.p., *Wangenheimia*) and 'broad-leaved' clade (*Drymochloa* (= *Festuca* subgen. *Drymanthele*), *Festuca* p.p., *Lolium*, *Micropyropsis*, *Schedonorus*) that have been studied by us (cf. Holub 1984, 1998; Watson & Dallwitz 1992). Despite the lack in taxon sampling for a few more Loliinae genera not included in the present survey (i.e. *Loliolum*, *Vulpiella*; cf. Clayton & Renvoize, 1986; Watson & Dallwitz 1992), it is still predictable that the fine-leaved clade would accumulate more traditionally recognized genera than the broad-leaved one. Torrecilla *et al.* (2004) interpreted the wide array of monotypic and small-sized genera described within the fine-leaved *Festuca* clade as a direct consequence of the higher speciation rates developed by these ephemeral groups. However, Gaut *et al.* (1997) found scant support for the speciation-rate hypothesis within the grass family except for the rapidly evolving Pooideae lineage that adjusted better to it. Our evolutionary test within Loliinae agrees with this partial result of Gaut *et al.* (1997).

The implications that the acquisition of the annual habit and the increase in ploidy might have had in the evolution of the festucoids cannot be conclusively established yet as taxon sampling is still incomplete for several groups of the large genus *Festuca* and its satellite genera. However, from the data analyzed here, it can be hypothetized with confidence that the two evolutionary scenarios (the MGT hypothesis and the reticulation/polyploidization hypothesis) are likely to have occurred at different evolutionary times within the subtribe Loliinae.

The switch from perennial towards annual life-cycle probably represents a more ancestral evolutionary phenomenon, independently experienced within the two main lineages of the festucoids as manifested in the mostly diploid nature of the ephemeral lineages. On the other hand, reticulate processes involving recurrent hybridization and polyploidy constitute secondary evolutionary events that have affected some of the most recently evolved perennial lineages of broad-leaved and fine-leaved *Festuca* and a few annual fine-leaved ones. Although accelerated rates are significantly different across the annual lineages, the polyploid complexes also show relatively higher rates, indicating that reticulation has fostered the subtitutional rates of these groups through the addition of new gene-pools.

Both scenarios agree, partly, with the speciation-rate hypothesis, as annuals and highly polyploid taxa show a wider array of taxa within them than their congeneric or cosectional relatives. However, whereas the annual lineages show distinctive traits that were used to classify them as different genera (Clayton & Renvoize 1986; Watson & Dallwitz 1992), the polyploid complexes are formed by a series of microtaxa that could hardly be morphologi-

cally differentiated from each other (Markgraf-Dannenberg 1980). These evidences add support for an older diversification of the annual lineages from their respective common ancestors and for a secondary and recent divergence of the polyploid lineages.

As a concluding remark, there is scope for speculation on future refinement of the present findings by characterising and subdividing the festucoid polyploid lineages into polyploid like-complexes when more detailed data become available. There is also increasing scope for the application of new chloroplast and nuclear genes to calculations of differences in substitution rates among Loliinae lineages in order to put the observed rate heterogeneity into a genome map context.

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REFERENCES

AINSCOUGH, M.M., BARKER C.M. & STACE C.A. (1986). Natural hybrids between *Festuca* and species of *Vulpia* section *Vulpia*. *Watsonia* **16**: 143–151.

ALEXEEV, E.B. (1977). K sistematike asiatskich ovsjaniz (*Festuca*). I. Podrod *Drymanthele*, *Subulatae*, *Schedonorus*, *Leucopoa* (To the systematics of Asian fescues (*Festuca*). I. Subgenera *Drymanthele*, *Subulatae*, *Schedonorus*, *Leucopoa*). *Bjulleten´ Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii* **82(3)**: 95–102.

ALEXEEV, E.B. (1978). K sistematike asiatskich ovsjaniz (*Festuca*). 2. Podrod *Festuca* (To the systematics of Asian fescues (*Festuca*). 2. Subgenus *Festuca*). *Bjulleten´ Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii* **83(4)**: 109–122.

ALEXEEV, E.B. (1980). Novye podrody i sekzii ovsjaniz (*Festuca* L.) Severnoj Ameriki i Meksiki (*Festuca* L. Subgenera et sectiones novae ex America boreali et Mexica). *Novosti Sistematiki Nizshikh Rastenii* **17**: 42–53.

ALEXEEV, E.B. (1981). Novye taksoni roda *Festuca* (Poaceae) is Meksiki i Zentral'noj Ameriki (The new taxa of the genus *Festuca* (Poaceae) from Mexico and Central America). *Botanicheskii ´Zhurnal* (Moscow and Leningrad) **66**: 1492–1501.

ALEXEEV, E.B. (1986). Ovsjanizy (*Festuca* L., Poaceae) Venezuely, Kolumbii i Ekvadora (*Festuca* L. (Poaceae) in Venezuela, Colombia et Ecuador). *Novosti Sistematiki Nizshikh Rastenii* **23**: 5–23.

BARKER, C.M. & STACE, C.A. (1982). Hybridization in the genera *Vulpia* and *Festuca*: the production of artificial F_1 plants. *Nordic J. Bot.* **2**: 435–444.

BARKER, C.M. & STACE, C.A. (1984). Hybridization in the genera *Vulpia* and *Festuca* (Poaceae): The characteristics of artificial hybrids. *Nordic J. Bot.* **4**(3): 289–302.

BARKER, C.M. & STACE, C.A. (1986). Hybridization in the genera *Vulpia* and *Festuca* (Poaceae): meiotic behaviours of artificial hybrids. *Nordic J. Bot.* **6**: 1–10.

BARRACOUGH, T.G. & SAVOLAINEN, V. (2001). Evolutionary rates and species diversity in flowering plants. *Evolution* **55**: 677–683.

BARRACLOUGH, T.G., HARVEY, P. & NEE, S. (1996). Rate of *rbc*L gene sequence evolution and species diversification in flowering plants (angiosperms). *Proc. R. Soc. Lond.* B **263**: 589–591.

BORRILL, M., KIRBY, M. & MORGAN, W.G. (1977). Studies in *Festuca*. 11. Inter-relationships of some diploid ancestors of the polyploid broad-leaved fescues. *New Phytol.* **78**: 661–674.

BOUSQUET, J., STRAUSS, S.H., DOERKSEN, A.H. & PRICE, R.A. (1992). Extensive variation in evolutionary rate of *rbc*L gene sequences among seed plants. *Proc. Natl. Acad. Sci. USA* **89**: 7844–7848.

CATALÁN P., TORRECILLA P., LÓPEZ-RODRÍGUEZ J.A. & OLSMTEAD R.G. (2004). Phylogeny of the festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and *trn*L_F sequences. *Mol. Phylogenet. Evol.* **31**: 517–541.

CHARMET, G., RAVEL, C. & BALFOURIER, F. (1997). Phylogenetic analysis in the *Festuca-Lolium* complex using molecular markers and ITS rDNA. *Theor. Appl. Genet* **94**: 1038–1046

CLAYTON, W.D. & RENVOIZE, S.A. (1986). Genera Graminum: Grasses of the World, *Kew Bull. Addit. series XIII*, Royal Botanic Gardens, Kew.

COTTON, R. & STACE, C.A. (1976). Taxonomy of the genus *Vulpia* (Gramineae). I. Chromosomes numbers and geographical distribution of the world species. *Genetica* **46**: 235–255.

COTTON, R. & STACE, C.A. (1977). Morphological and anatomical variation of *Vulpia* (Gramineae). *Bot. Notiser* **130**: 173–187.

DARBYSHIRE, S.J. & WARWICK, S.I. (1992). Phylogeny of North American *Festuca* (Poaceae) and related genera using chloroplast DNA retriction site variation. *Canad. J. Bot.* **70**: 2415–2429.

DUMORTIER B.C.J. (1823). *Observations sur les Graminées de la flore Belgique. J. Casterman. Tournay*. Belgium.

EYRE-WALKER, A. & GAUT, B.S. (1997). Correlated rates of synonymous site evolution across plant genomes. *Mol. Biol. Evol.* **14**: 455–460.

GAUT, B.S., CLARK, L.G., WENDEL, J.F. & MUSE, S.V. (1997). Comparisons of molecular evolutionary process at *rbc*L and *ndh*F in the grass family (Poaceae). *Molec. Biol. Evol.* **14**: 769–777.

GAUT, B.S., MUSE, S.V., CLARK, W.D. & CLEGG, M.T. (1992). Relative rates of nucleotide substitutions at the *rbc*L locus of monocotyledonous plants. *J. Mol. Evol.* **35**: 292–303.

GAUT, B.S., MORTON, B.R., MCCAIG, B.C. & CLEGG, M.T. (1996). Susbstitution rate comparisons between grasses and palms: synonymous rate differences at the nuclear gene *Adh* parallel rate differences at the plastid gene *rbc*L. *Proc. Natl. Acad. Sci. USA* **93**: 10274–10279.

GAUT, B.S., TREDWAY, L.P., KUBIK, C., GAUT, R.L. & MEYER, W. (2000). Phylogenetic relationships and genetic diversity among members of the *Festuca-Lolium* complex (Poaceae) based on ITS sequence data. *Pl. Syst. Evol.* **224(1-2)**: 33–53.

GIVNISH T.J. (1997). Adaptative radiation and molecular systematics: Issues and approaches. In: GIVNISH T.J. & SYSTMA K.J. (eds), *Molecular evolution and adaptive radiation*. Pp. 1–54. Cambridge University Press. Cambridge. UK.

GMELIN, C.C. (1805). *Flora badensis alsatica* . Vol. 1, Müller, Karlsruhe.

HACKEL, E. (1882). *Monographia* Festucarum *Europearum*, Theodor Fischer, Kassel und Berlin, pp. 216, 4 lam.

HACKEL, E. (1887). Gramineae. In: H.G.A. ENGLER & K.A.E. PRANTL, (eds), *Die Natürlichen Pflanzenfamilien*, Teil 2, Abteilung 2, Engelmann, Leipzig, pp. 1–97.

HACKEL, E. (1906). Gramineae novae. *Repertorium Novarum Speciarum Regni Vegetabilis* **2**: 69–72.

HARPER, J.A., THOMAS, I.D., LOVATT, J.A. & THOMAS, H.M. (2004). Physical mapping of rDNA sites in posible diploid progenitors of polyploid *Festuca* species. *Pl Syst Evol* **245**: 163–168.

HILLIS, D.M. & WIENS, J.J. (2000). Molecules versus Morphology in Systematics. Conflicts, Artifacts, and Misconceptions. In: J.J. WIENS (ed.), *Phylogenetic Analysis of Morphological Data*, Smithsonian Series in Comparative Evolutionary Biology. Smithsonian Institution Press, Washington., pp. 1–19.

HOLUB, J. (1984). New Genera in Phanerogamae [1–3]. *Folia Geobotanica et Phytotaxonomica* **19**: 95–99.

HOLUB, J. (1998). Reclassifications and new names in vascular plants 1. *Preslia* **70**: 97–122.

HUELSENBECK, J. P. & RONQUIST, F. (2002). MRBAYES. Bayesian inference of phylogenetic trees. V. 3.0. *Bioinformatics* **17(8)**: 754–755.

HUMPHREYS, M.W., THOMAS, H.M., MORGAN, W.G., MEREDITH, M.R., HARPER, J.A., THOMAS, H., ZWIERZYKOWSKI, Z. & GUESUIéRE, M. (1995). Discriminating the ancestral progenitors of hexaploid *Festuca arundinacea* using genomic in situ hybridisation. *Heredity* **75**: 171–174.

JAHUAR, P.P. (1975). Genetic regulation of diploid-like chromosome pairing in the hexaploid species *Festuca arundinacea* Schreb. and *F. rubra* L. (Gramineae). *Chromosoma* **52**: 363–382.

JAHUAR, P.P. (1993). Cytogenetics of the *Festuca-Lolium* complex 1993; Berlin Heidelberg: Springer.

JENKIN, T.J. (1933). Interspecific and intergeneric hybrids in herbage grasses. Initial crosses. *Journal of Genetics* **28**: 205–264.

KRECHETOVICH, V.I. & BOBROV, E.G. (1934). *Festuca* L. s. str. In: V.L. KOMAROV, R.Y. ROZHEVITS & B.K. SHISHKIN (eds), *Flora SSSR*. Vol. 2, Akademija Nauk SSSR, Leningrad, pp. 497–535.

KRIVOTULENKO, U. (1960). Novye sekzii roda *Festuca* L. (Generis *Festuca* L. sectiones novae). *Bot. Mat. (Leningrad)* **20**: 48–67.

LEHVÄSLAIHO, H., SAURA, A. & LOKKI, J. (1987). Chloroplast DNA variation in the grass tribe Festuceae. *Theor. Appl. Genet.* **74**: 298–302.

LEWIS, E.J. (1975). *Festuca* L. × *Lolium* L. = × *Festulolium* Aschers. & Graebner. In: C.A. STACE (ed.), *Hybridization and the flora of the British Isles*, pp. 547–552.

LI, P. & BOUSQUET, J. (1992). Relative-rate test for nucleotide substitutions between two lineages. *Molec. Biol. Evol.* **9**: 1185–1189.

MALIK, C.P. & THOMAS, P.T. (1966). Karyotypic studies in some *Lolium* and *Festuca* species. *Caryologia* **19**: 167–196.

MARKGRAF-DANNENBERG, I. von. (1980). *Festuca* L. Pp. 125–153. In: T.G. TUTIN *et al.* (eds), *Flora Europaea*, Vol. 5. Cambridge University Press, Cambridge, U.K.

MUSE, S.V. (2000). Examining rates and patterns of nucleotide substitutions in plants. *Pl. Mol. Biol.* **42**: 25–43.

PIPER, C.V. (1906). North American species of *Festuca*. *Contributions from the United States National Herbarium* **10**: 1–48.

POSADA, D. & CRANDALL, K.A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14(9)**: 817–818.

ROBINSON, M., GOUY, M., GAUTIER, C. & MOUCHIROUD, D. (1998). Sensitivity of the relative-rate test to taxonomic sampling. *Mol. Biol. Evol.* **15**: 1091–1098.

ROBINSON-RECHAVI, M. & HUCHON, D. (2000). RRTree: Relative-Rate Test between group of sequences on a phylogenetic tree. *Bioinformatics* **16**: 296–297.

SANDERSON, M.J. (1997). A nonparametric approach to estimating divergence times in the absence of rate constancy. *Mol. Biol. Evol.* **14**: 1218–1231.

SCHOLZ, H., STIERSTORFER, C. & GAISBERG, M.V. (2000). *Lolium edwardii* sp. nova (Gramineae) and its relationship with *Schedonorus* sect. *Plantynia* Dumort. *Feddes Repertorium* **111**: 561–565.

SOLTIS, D.E. & SOLTIS, P.S. (1999). Polyploidy: recurrent formation and genome evolution. *TREE* **14**: 348–352.

SORENG, R.J. & DAVIS, J.I. (2000). Phylogenetic structure in Poaceae subfamily Pooideae as inferred from molecular and morphological characters: misclassification versus reticulation. In: S.W.L. JACOBS & J. EVERETT (eds), *Grasses: Systematics and Evolution*, CSIRO, Melbourne, pp. 61–74.

SORENG, R.J. & TERRELL, E.E. (1998). Taxonomic notes on *Schedonorus*, a segregate genus from *Festuca* or *Lolium*, with a new nothogenus, ×*Schedololium*, and new combinations. *Phytologia* **83**: 85–88.

STACE, C.A. (1981). Generic and infrageneric nomenclature of annual Poaceae: Poeae related to *Vulpia* and *Desmazeria*. *Nordic J. Bot.* 1: 17–26

STACE, C.A. (1987). Hybridization and the plant species. In: K.M. URBANSKA (ed.), *Differentiation patterns in higher plants*, pp. 115–127. Academic Press, London.

STEBBINS, G.L. (1956). Cytogenetics and evolution of the grass family. *Am. J. Bot.* **43**: 890–905.

SUZUKI, J.I, HERBEN, T., KRAHULEC, F. & HARA, T. (1999). Size and spatial pattern of *Festuca rubra* genets in a mountain grassland: its relevance to genet establishment and dynamics. *J. Ecol.* **87**: 942–954.

TERRELL, E.E. (1968). *A taxonomic revision of the genus Lolium*. U.S. Dept. Agric. Tech. Bull. 1392.

TORRECILLA, P. & CATALÁN, P. (2002). Phylogeny of broad-leaved and fine-leaved *Festuca* linages (Poaceae) based on nuclear ITS sequences. *Syst. Bot.* **27(2)**: 241–251.

TORRECILLA, P., LÓPEZ-RODRÍGUEZ, J.A., STANCIK, D. & CATALÁN, P. (2003). Systematics of *Festuca* sects. *Eskia* Willk., *Pseudatropis* Kriv., *Amphigenes* (Janka) Tzvel., *Pseudoscariosa* Kriv., and *Scariosae* Hack. based on analysis of morphological characters and DNA sequences. *Pl. Syst. Evol.* **239**: 113–139.

TORRECILLA, P., LÓPEZ-RODRÍGUEZ, J.A. & CATALÁN, P. (2004). Phylogenetic relationships of *Vulpia* and related genera (Poeae, Poaceae) based on analisis of ITS and *trn*L-F sequences. *Ann. Missouri Bot. Gard.* **91**: 124–158.

TZVELEV, N.N. (1971). K sistematike i filogenii ovsjaniz (*Festuca* L.) flory SSSR. I. Sistema roda i oshoriye naprav'enija evoljuzii.. (On the taxonomy and phylogeny of genus *Festuca* L. of the U.S.S.R. flora. I. The system of the genus and main trends of evolution). *Botanicheskii Zhurnal (Moscow and Leningrad)* **56**: 1252–1262.

TZVELEV, N.N. (1982). Poryadok zlaki (Poales). *Zhizn Rast.* **6**: 341–378.

TZVELEV, N.N. (1999). Ob obeme I nomenklature nekotorykh rodov sosudistykh rastenii Evropeiskoi Rossii. (On the size and nomenclature of some genera of the vascular plants of European Russia). *Bot. Zhurn.* **84(7)**: 109–118.

TZVELEV, N.N. (2000). Combinationes novae taxorum plantarum vascularium. *Novit. Syst. Pl. Vasc.* **32**: 181–183.

WATSON, L. & DALLWITZ, M.J. (1992). *The Grass Genera of the World.* C.A.B. International. Cambridge University Press. Cambridge.

WENDEL, J.F. & DOYLE, J.J. (1998). Phylogenetic incongruence: window into genome history and molecular evolution. In: D.E. SOLTIS, P.S. SOLTIS & J.J. DOYLE (eds), *Molecular Systematics of Plants II. DNA Sequencing*, Kluwer Academic, Boston, pp. 265–296.

WU, C.I. & LI, W.H. (1985). Evidence for higher rates of nucleotide substitution in rodents than in man. *Proc. Acad. Natl. Sci. USA* **82**: 1741–1745.

XU, W.W. & SLEPER, D.A. (1994). Phylogeny of tall fescue and related species using RFLPs. *Theor Appl Gen* **88**: 685–690.

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Bailey, J. & Ellis, R.G. (eds) 71–88 (2006), BSBI. London.

What use is sex?

Rock sea-lavenders (*Limonium binervosum* agg.) revisited

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ABSTRACT

Analysis of morphometric variation within and between populations of *Limonium binervosum* agg., rock sea-lavender has revealed extensive, statistically significant, differentiation between populations of the same infra-specific taxon, even those growing in close geographical vicinity. Morphological groups are largely supported by patterns of amplified DNA fragment length polymorphisms but genetical distances contrast markedly from phenetic/morphological distances. It is recommended that a more informal and simplified classification of the group is utilised. The evolution of the obligately asexual group is discussed and it is suggested that much of the observed pattern of variation may be due to heritable epigenetic differences.

Keywords: Geographical variation, apomict, epigenetic, evolution.

INTRODUCTION

Limonium binervosum agg., the rock sea-lavender, is a complex of agamospermous taxa that grows on the coastal cliffs and at the margins of salt-marshes and shingle banks in the British Isles and western Europe from northern France to southern Portugal. In the British Isles there are four widespread and five narrow endemic species (Table 1). In addition infra-specific taxa at subspecific and varietal rank have been described, each also confined to a greater or lesser extent to clearly defined stretches of coastline (Ingrouille & Stace 1986), for example the subspecies of *L. recurvum* illustrated in Fig. 1. The narrow endemics are confined to a short stretch of coast or even a single headland but have a markedly different morphology from adjacent populations, such as the clustered spike of *L. paradoxum* (Fig. 2).

This complex hierarchical classification was designed to summarise a hierarchical pattern of relationships between major and minor variants (Ingrouille & Stace 1985) that was discovered from a taxometric survey of 153 native populations carried out between 1979 and 1981 (Ingrouille 1982). The relationships were discovered by multivariate statistical analysis of morphological characters measured mainly in plants cultivated under uniform conditions in the University of Leicester Botanic Gardens. Morphological characters from both the vegetative and flowering parts of the plant were measured. Cluster analysis and principal components analysis of populations was used to discover groups of populations and clusters were validated by comparison with other characters not included in the multivariate analyses, such as pollen morphology, ecology, chromosome number and most importantly geographical distribution.

TABLE 1. TAXA OF *LIMONIUM BINERVOSUM* AGG. NATIVE TO THE BRITISH ISLES

Widespread species

L. binervosum (G.E.Sm.) C.E.Salmon	*L. procerum* (C.E.Salmon) Ingr.
subsp. *binervosum*	subsp. *procerum* Ingr.
subsp. *mutatum* Ingr.	var. *procerum*
subsp. *anglicum* Ingr.	var. *medium* Ingr.
subsp. *saxonicum* Ingr.	var. *hibernicum* Ingr.
subsp. *cantianum* Ingr.	var. *cornubiense* Ingr.
subsp. *sarniense* Ingr.	var. *paramedium* Ingr.
var. *sarniense*	var. *wessexense* Ingr.
var. *aurigniense* Ingr.	subsp. *devoniense* Ingr.
var. *sercquense* Ingr.	subsp. *cambrense* Ingr.
L. britannicum Ingr.	*L. recurvum* C.E.Salmon
subsp. *britannicum*	subsp. *recurvum*
var. *britannicum*	subsp. *portlandicum* Ingr.
var. *kelseyanum* Ingr.	var. *portlandicum*
subsp. *coombense* Ingr.	var. *recurviforme* Ingr.
var. *coombense*	var. *kerryense* Ingr.
var. *grandicaule* Ingr.	subsp. *pseudotranswallianum*
subsp. *transcanalis* Ingr.	subsp. *humile* (Girard) Ingr.
subsp. *celticum* Ingr.	var. *humile*
var. *celticum*	var. *donegalense* Ingr.
var. *pharense* Ingr.	var. *pseudoparadoxum* Ingr.

Narrow endemics

L. transwallianum (Pugsley) Pugsley	*L. loganicum* Ingr.
L. paradoxum Pugsley	*L. parvum* Ingr.
var. *paradoxum*	*L. dodartiforme* Ingr.
var. *mutabile* Ingr.	

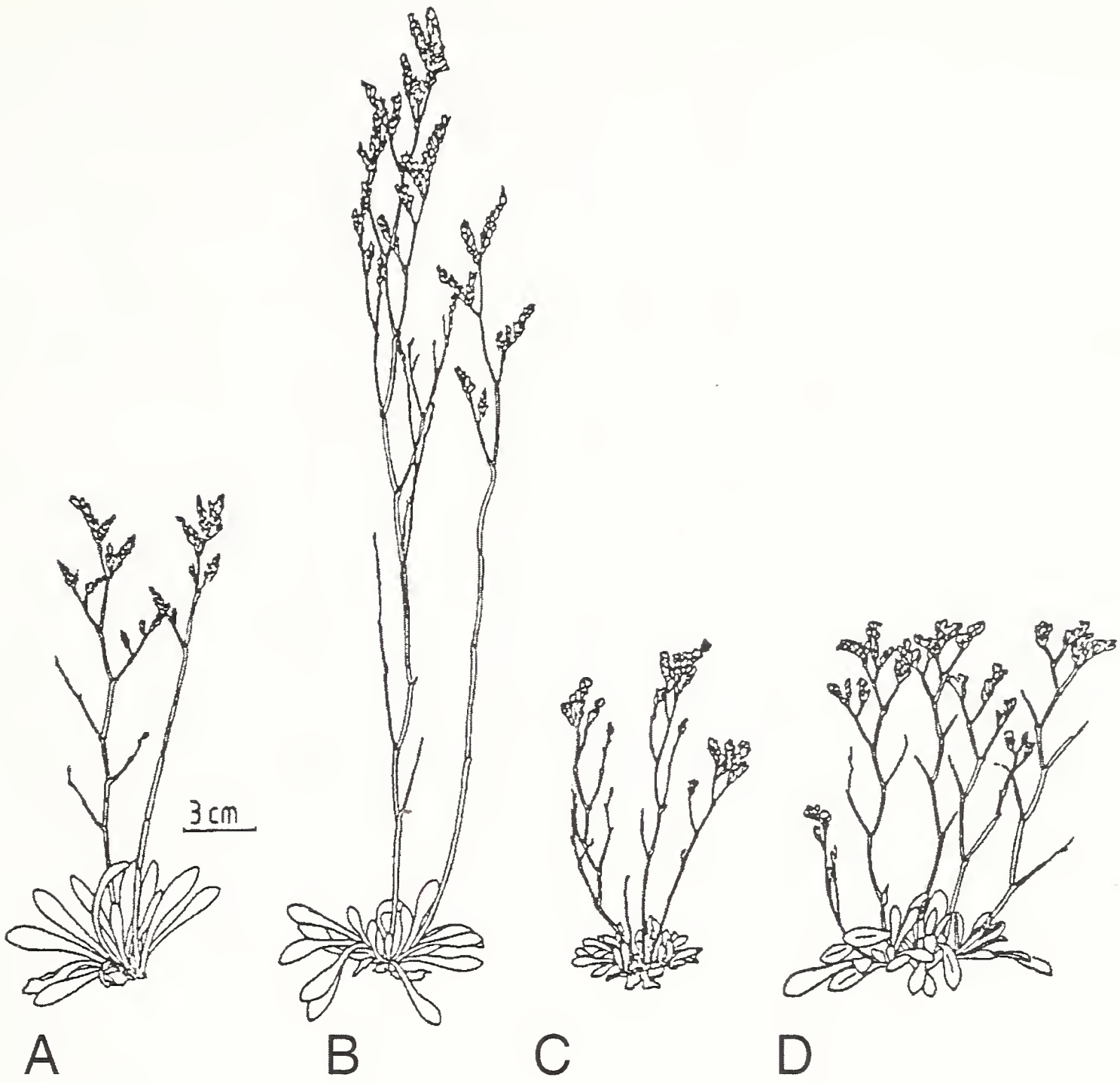


FIGURE 1. Variants of *L. recurvum*: A. subsp. *recurvum*, B. subsp. *portlandicum*; C. subsp. *pseudotranswallianum*; D. subsp *scoticum*

It is now nearly 30 years since this work and it is timely to evaluate the success or failure of the classification.

1 Although the classification has been incorporated in the *New Flora of the British Isles* (Stace 1997) to subspecific rank it has not proved practicable. Botanists have found it difficult to identify plants, especially if they are from populations not included originally in the 1979-81 survey.

2 In an unpublished survey of *L. binervosum* agg. on mainland of Europe carried out by the author in the 1990s it proved difficult to incorporate European taxa (*L. dodartii* and *L. multiflorum*) and other distinct but undescribed variants within the classification that had been designed for the British Isles.

3 Although only a small amount of molecular data has been obtained it has in some cases indicated quite different patterns of variation (Cowan *et al.* 1998). Analysis of amplified fragment polymorphisms has shown some taxa to be more and other taxa to be less genetically distinct than expected from their morphology. For example the subspecies *L. binervosum* subsp. *anglicum* is as distinct as any species within the aggregate and the narrow endemic species *L. parvum*, *L. paradoxum* and *L. transwallianum* that are very distinct morphologically are very close to other taxa (Fig. 3).

FIGURE 2. Variants of the narrow endemic *L. paradoxum* from Saint David's Head (v.c. 45) in Pembrokeshire differing in spike morphology and chromosome number

These difficulties raise several questions about the original study and the way the results were translated into a revised classification. At that time the computer based methods for multivariate analysis of large datasets were limited and populations rather than individual plants were utilised as the smallest operational taxonomic unit. The use of population means obscured any within population variation present, though it was thought at the time from direct observation in nature, that there was little intra-population variation. This impression was exacerbated by the apparent homogeneity of a small sub-sample of wild populations (no more that 10 plants sampled as cuttings from rosettes) that was cultivated in the botanic garden.

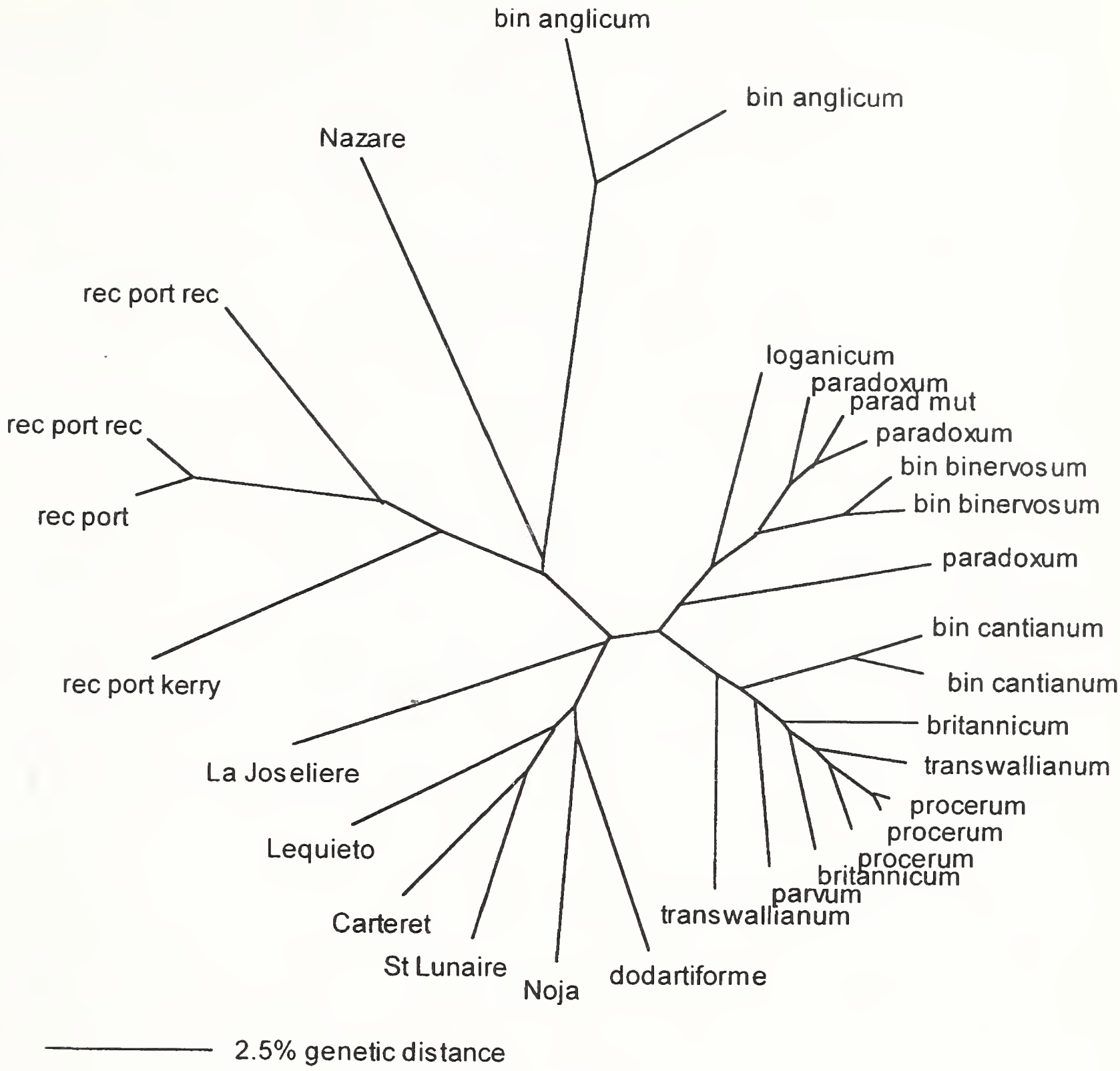


FIGURE 3. Tree (created by unweighted paired group method analysis (UPGMA) of genetic distances measured from amplified DNA fragment polymorphisms (AFLPs)): acronyms relate to taxonomic names (see Table 1) except for the names of sites of populations on continental Europe. Note the separation of *L. binervosum* subsp. *anglicum* ('bin anglicum') from other taxa, and the inclusion of narrow endemics (*L. loganicum*, *L. paradoxum* with other *L. binervosum* and *L. parvum* and *L. transwallianum* with *L. procerum*/*britannicum*)

Originally, as well as 41 metric characters a large number of 'shape' characters calculated from ratios of the metric characters were utilised. This is no longer recommended because of the possibility of biasing the results if significant correlations between measurements exist. In the original analysis great weight was given to the results of a cluster analysis but this may have exaggerated the distinction between variants. Plants were cultivated before they were scored and while analysis of cultivated plants reduced potentially confounding environmentally caused variation, the results may not have properly represented genotypic variation as expressed in nature.

A number of more fundamental questions are also relevant.

- What is the relationship of morphological data to other kinds of data and which, if any, should have primacy in developing classifications?
- How can taxonomic rank be determined where data on breeding behaviour is not available, as in an asexual group like *L. binervosum* agg.?
- Is it always possible to create a workable classification for the non-expert that properly reflects natural relationships?
- What taxonomic rank, if any should be used for agamospermous variants?
- What is the value of taxonomically naming asexual variants?

With the questions listed above in mind it was thought worthwhile to re-analyse data collected in the original survey.

MATERIALS AND METHODS

146 populations sampled (81 from both cultivated and wild plants, 57 from cultivated only and 8 from wild only) with up to 10 individuals from each population. 41 metric characters measured. More than 2240 individuals were measured for 18 floral and spikelet characters and about half that number also for scape and leaf characters (an additional 23 characters) (Ingrouille 1982). Multivariate analysis was carried out utilising SPSS for Windows. Principal Components Analysis and Discriminant Analysis were carried out both on population means and individuals of all species and separately on individuals of pairs of species.

RESULTS

The results of the principal components analyses emphasise the close relationship of species. The narrow endemic species form distinct clusters at the margins of the distribution of individuals of the widespread taxa (Fig. 4). Principal components analysis of individuals of the widespread species provides some support for the existence of the four major clusters/species (Fig. 5). Discriminant analysis, with species as the discriminator, effectively separates the species even when plants are analysed rather than populations (Fig. 6). Discriminant analysis of the four widespread species including each pair of species one at a time is very effective at separating the species. If only 18 floral characters are used the degree of overlap between species is greater than if all 41 possible characters are utilised (Table 2) especially in a discriminant analysis of *L. procerum* and *L. britannicum* (Fig. 7).

From the percentage of miss-classified individuals shown in Table 2 it is clear that the two closest widespread species are *L. britannicum* and *L. procerum*. They are close in floral and spike characters but *L. britannicum* differs from *L. procerum* in its smaller stature and leaf shape.

Similarly discriminant analysis separates intraspecific taxa. For example the subspecies of *L. binervosum sensu stricto* in SE England are clearly separated (Fig. 8). *L. binervosum* subsp. *saxonicum* is the most distinct and *L. binervosum* subsp. *anglicum* and *L. binervosum* subsp. *binervosum are* closest to each other.

Analysis of individuals reveals unsuspected inter-population variation even within named infra-specific taxa. For example a discriminant analysis of the populations of *L. britannicum* subsp. *britannicum* from the N Cornwall coast (Fig. 9) separates several of these. Only one distinct population at Kelsey Head has been previously recognised as a

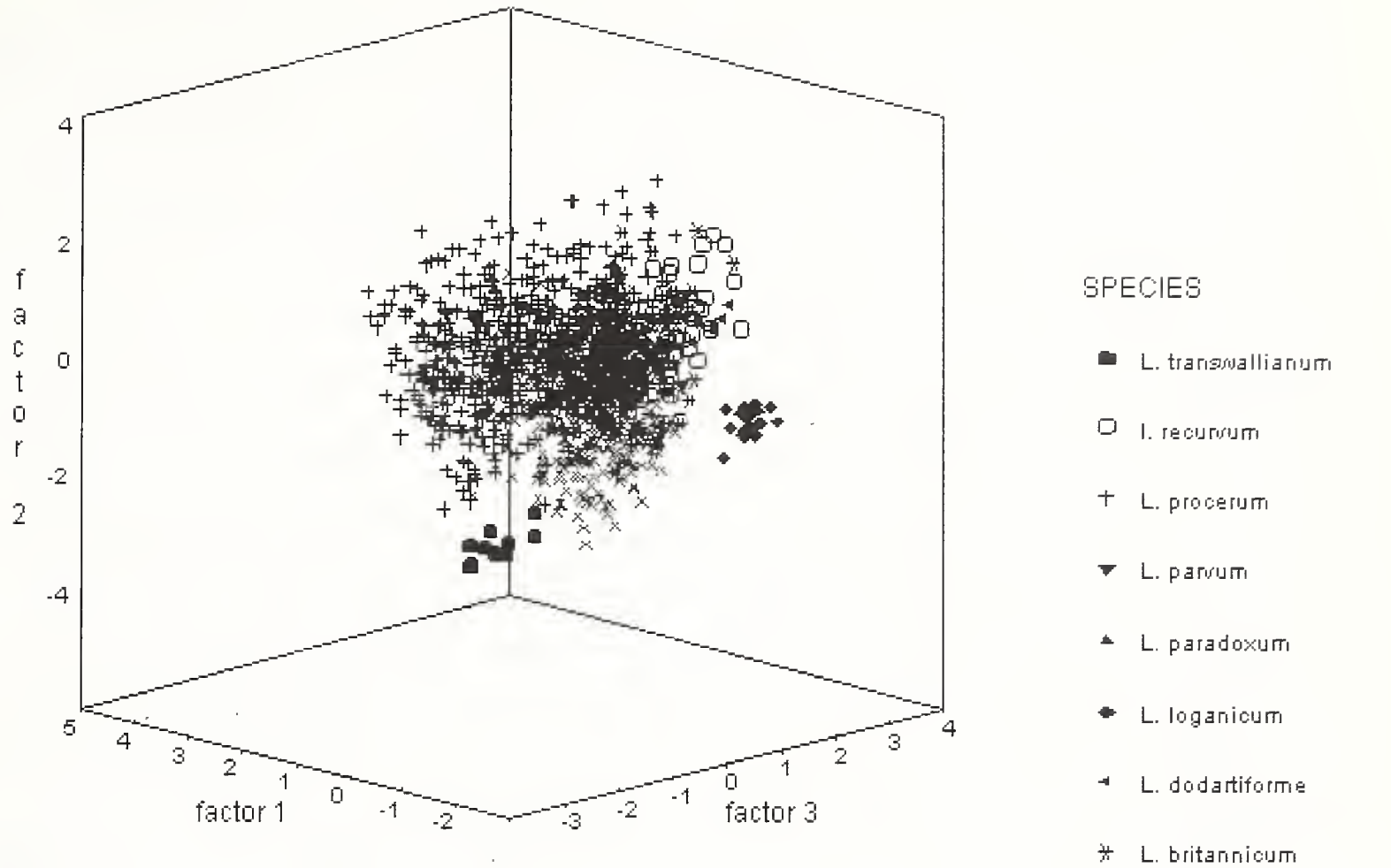


FIGURE 4. Three dimensional scatter-plot of the first three factor scores from a principal components analysis of individuals of all species. Note the position of *L. loganicum* and *L. transwallianum* on the margins of the main cluster. Other views and plots of different combinations of factors similarly isolates the other narrow endemic species.

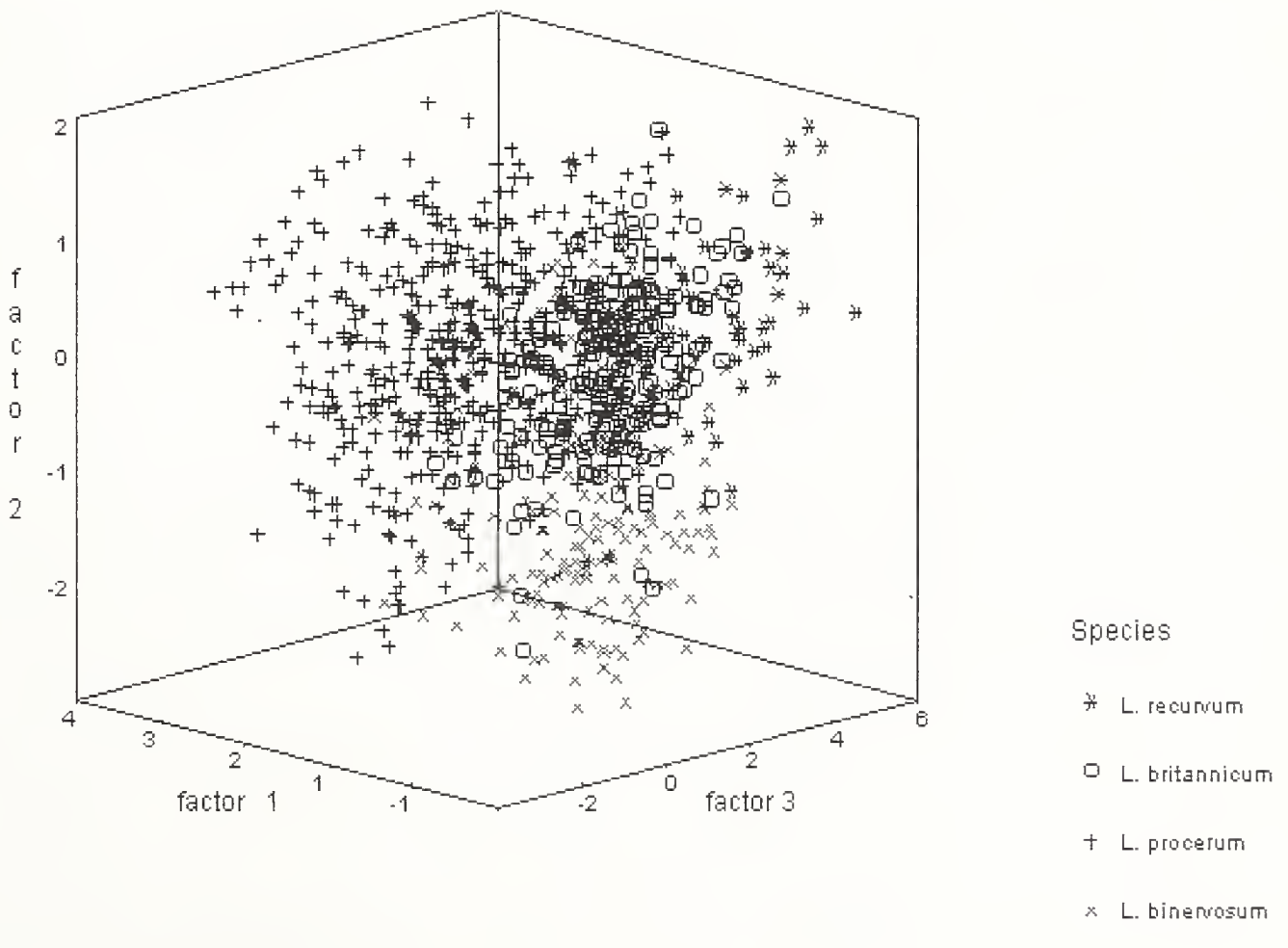


FIGURE 5. Three dimensional scatter-plot of the first three factor scores from a principal components analysis of individuals of the four widespread species with each occupying a different area of the main plot.

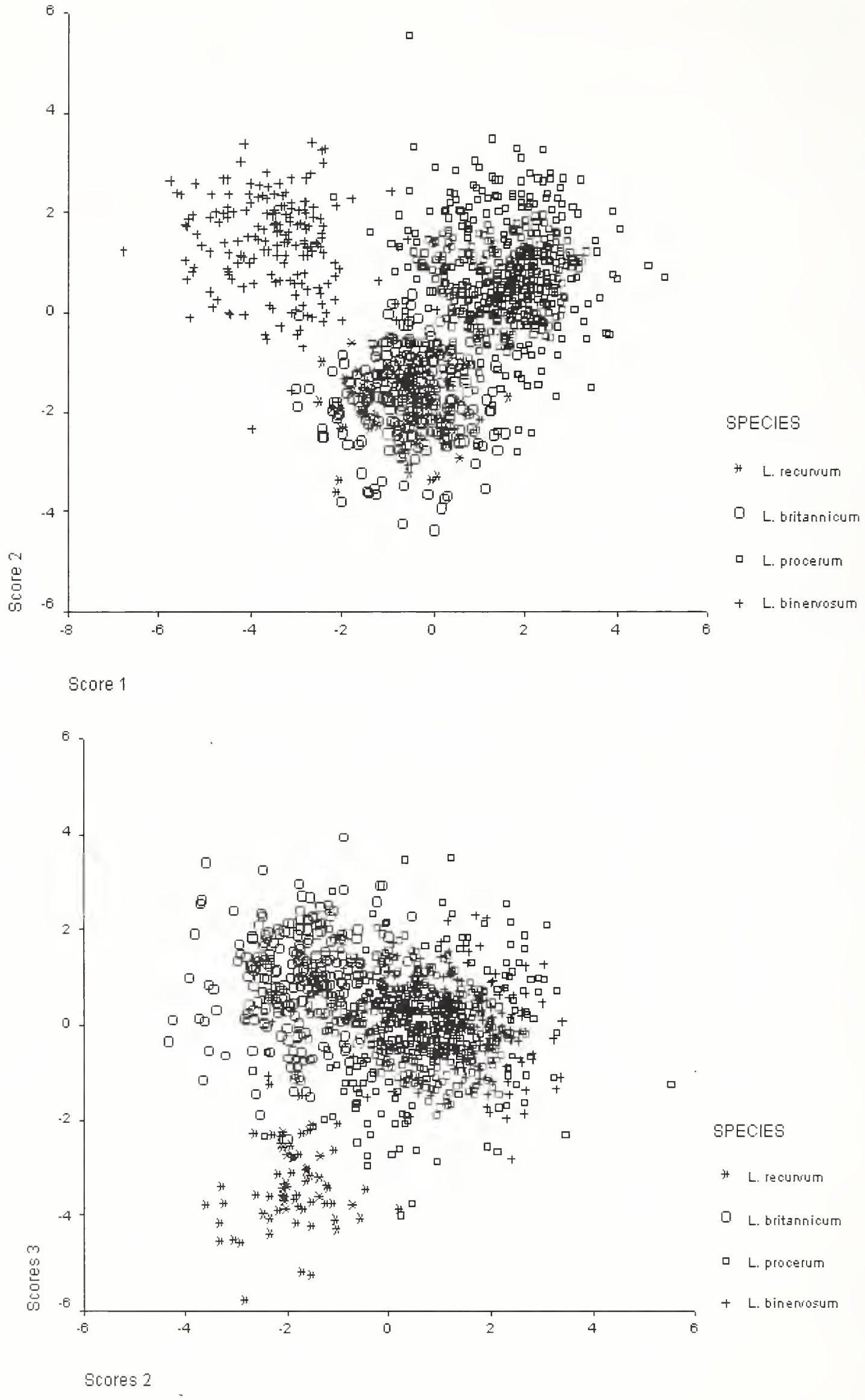


FIGURE 6. Two-dimensional scatterplots of discriminate scores from an analysis of the four widespread species : A (upper). Scores 1 and 2 showing the separation of *L. procerum*, *L. binervosum* and *L. britannicum*/*L. recurvum*; B (lower). Scores 2 & 3 showing separation of *L. recurvum*, *L. britannicum* and *L. procerum*/*L. binervosum*.

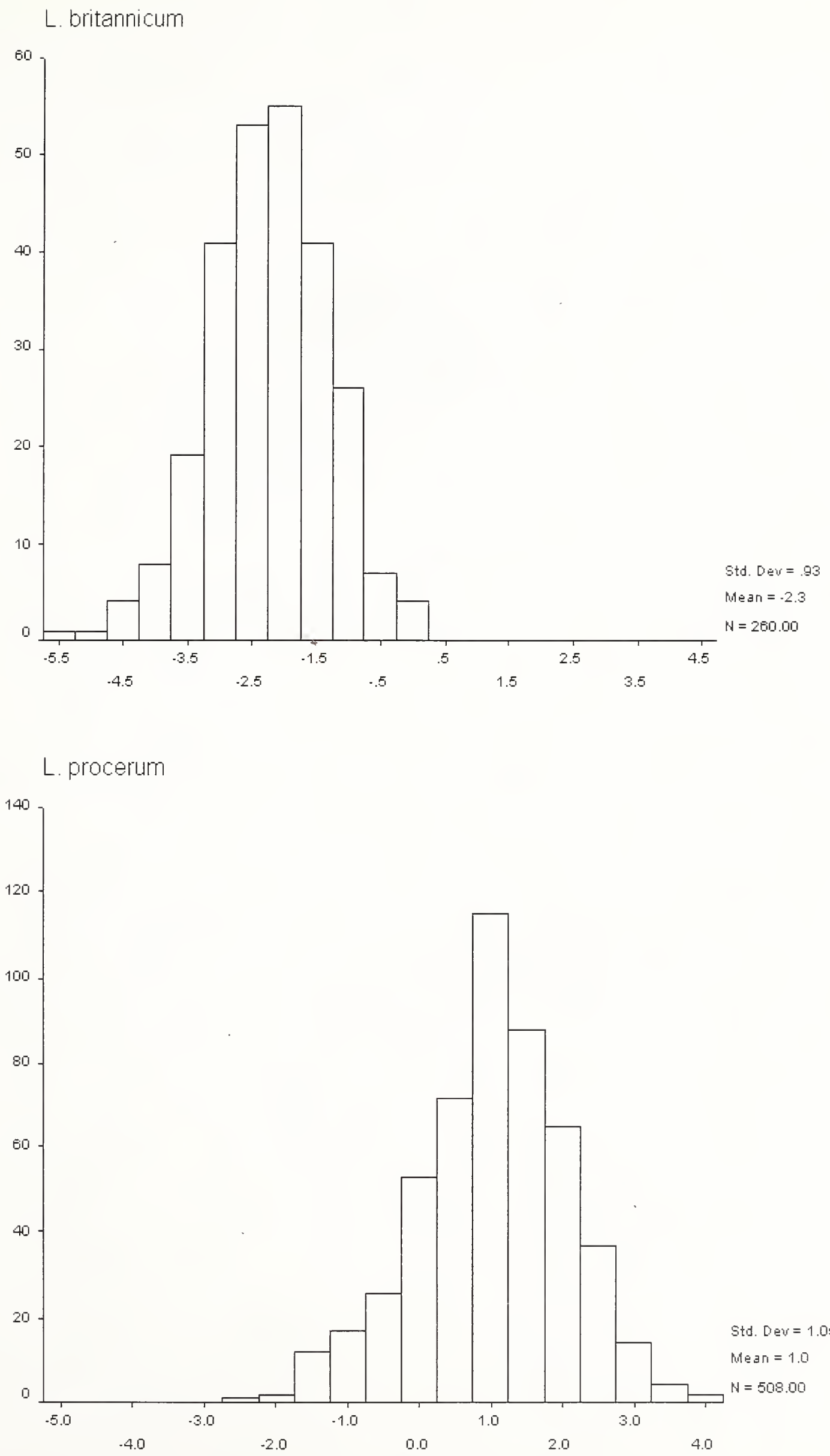
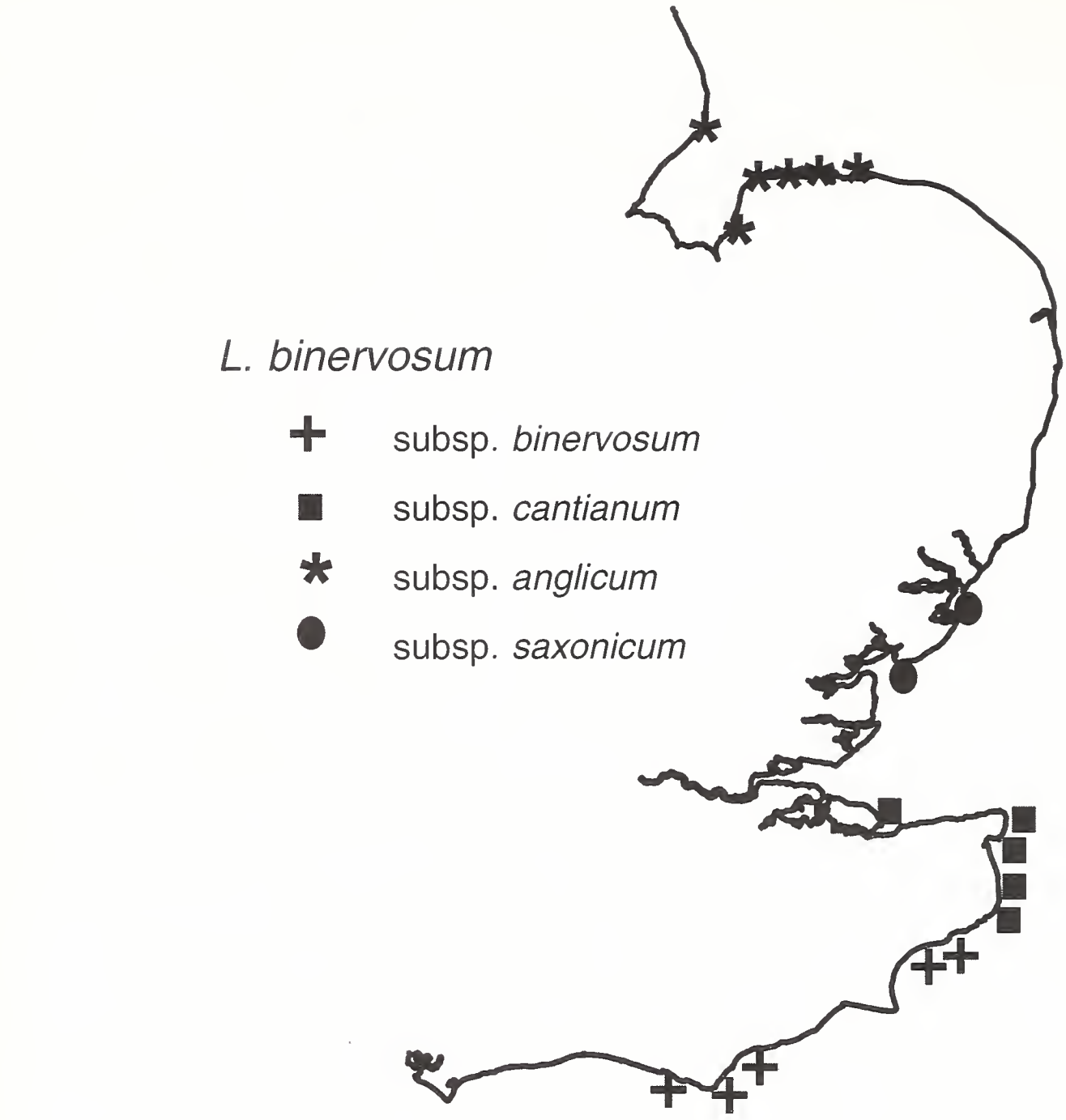


FIGURE 7. Histograms of the discriminant score from a discrimant analysis of *L. britannicum* and *L. procerum*.



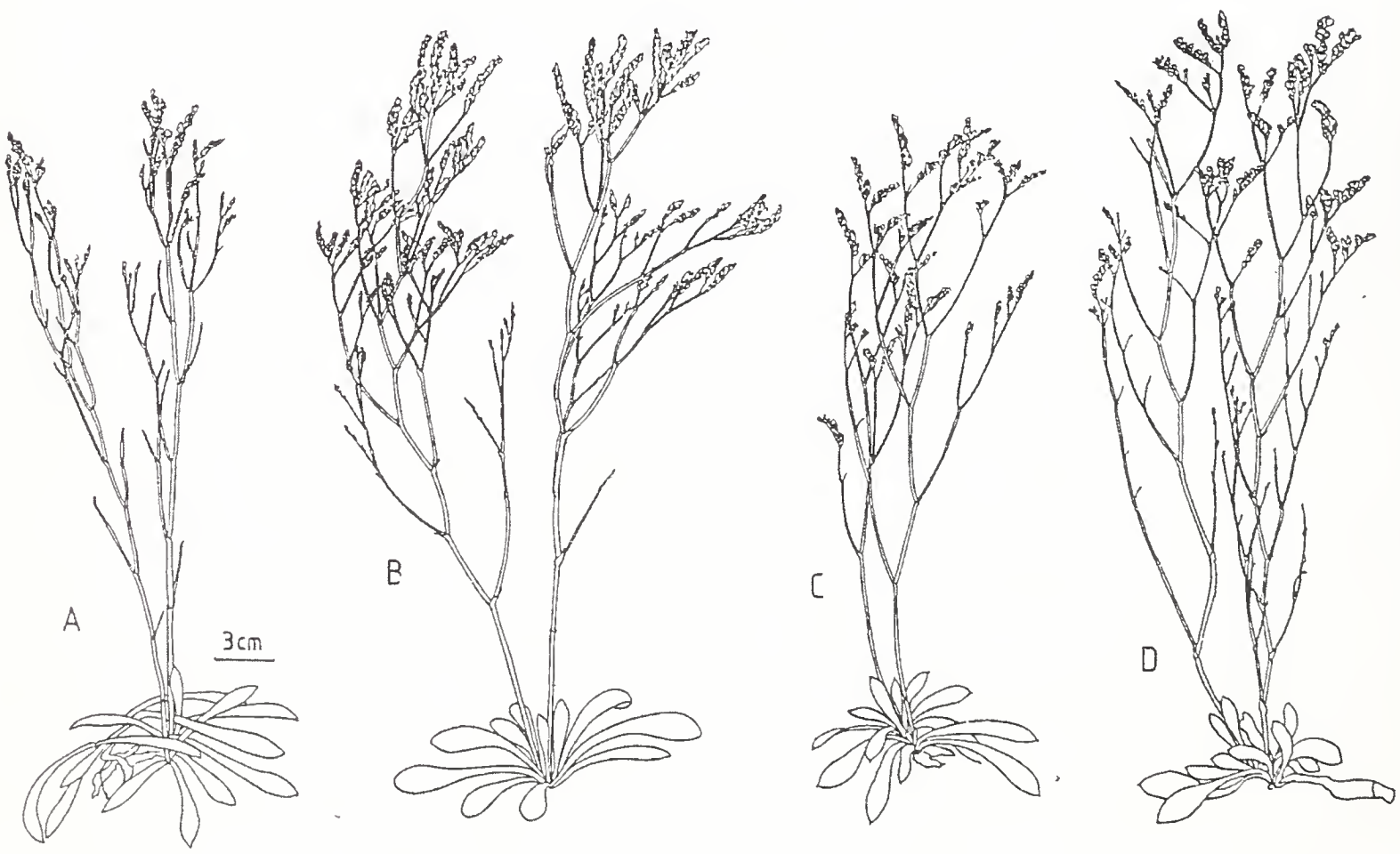


FIGURE 8. *L. binervosum sensu stricto* from SE England: distribution of infraspecific taxa A. subsp. *binervosum*, B. subsp. *cantianum*, C. subsp. *anglicum*, D. subsp. *saxonicum*, and separation of taxa by a discriminant analysis (overleaf).

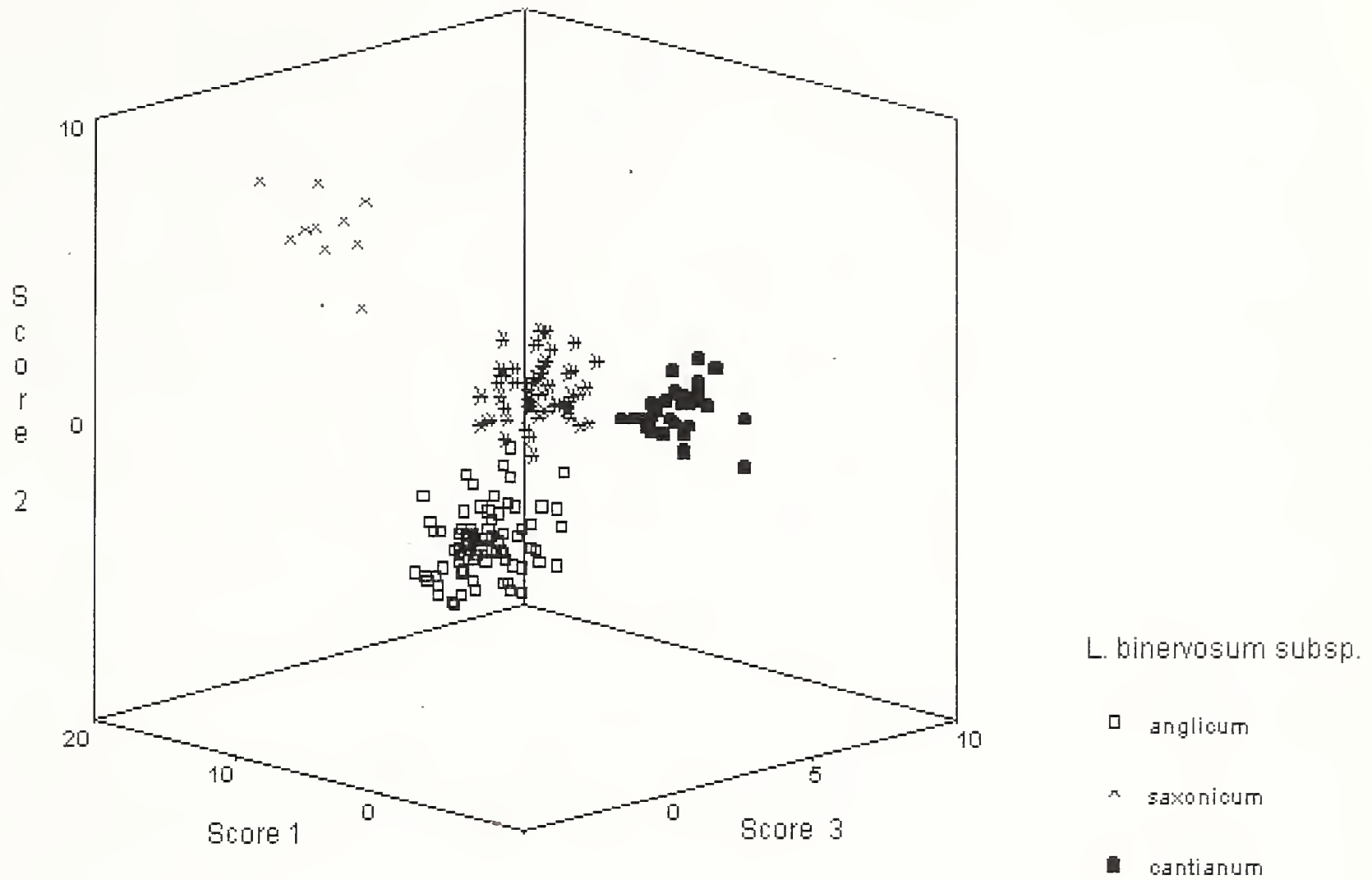
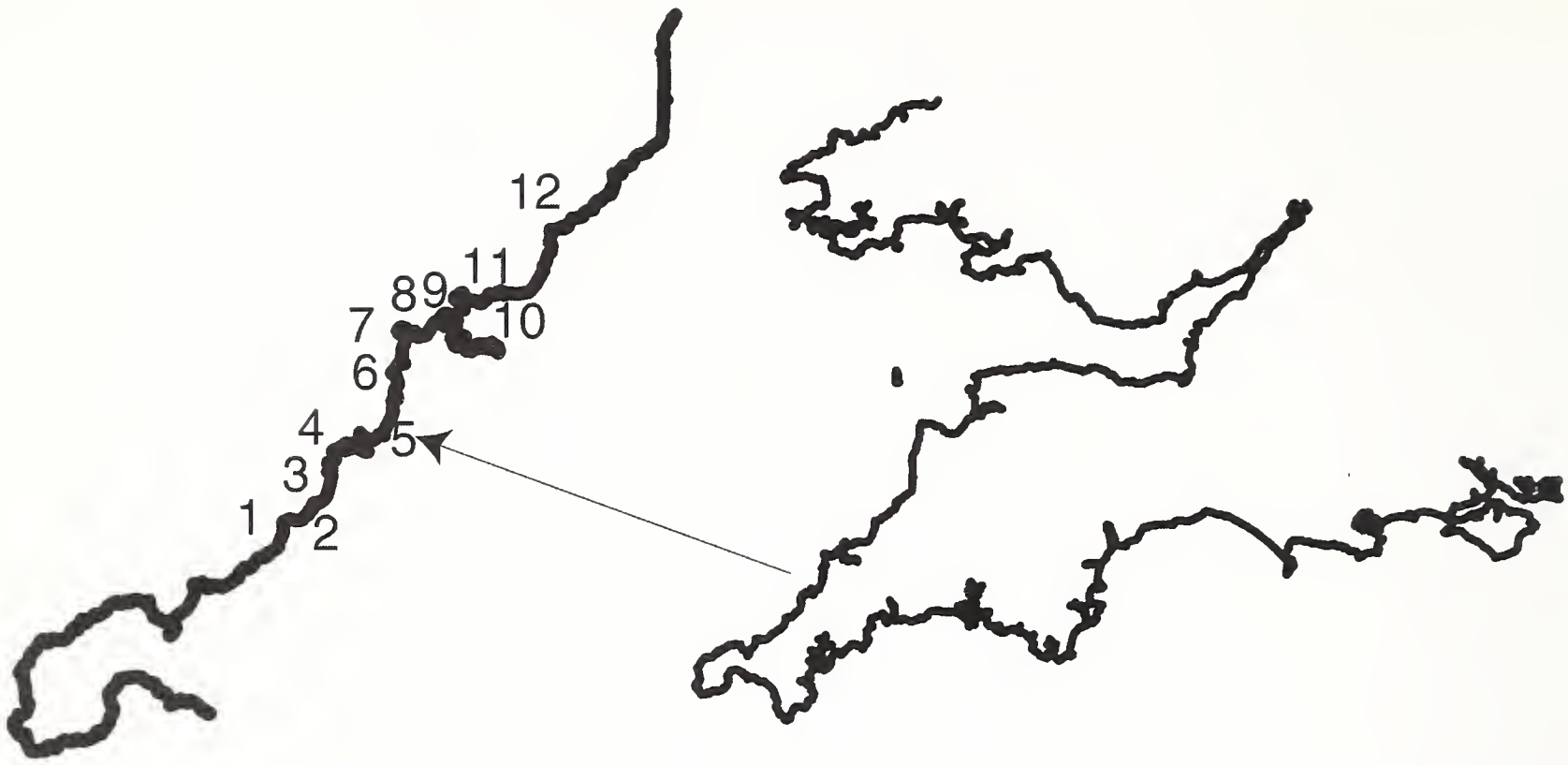


FIGURE 8. *L. binervosum sensu stricto* from SE England: separation of infraspecific taxa by a discriminant analysis.

distinct variety because of its smaller flowers. Another example is provided by the plants from populations of *L. binervosum* subsp. *sarniense* in the Channel Isles (Fig. 10). Again several populations form distinct clusters in a scatter plot of discriminant scores even though only two populations were formally recognised as distinct (SACC with decumbent scapes = var. *aurigiense*, and the dwarf SSVP = var. *sercquense*.). Analysis of variance can find many significant differences between these and other populations. Other similar examples could have been provided from *L. recurvum* on the Isle of Portland in Dorset or western Ireland and *L. procerum* in N Wales.

DISCUSSION

Reanalysis of the data utilising in individuals as operational taxonomic units (OTUs) challenges what was previously understood about the *L. binervosum* aggregate in two ways: firstly the taxonomic treatment; and secondly what is understood about the evolution of the group.



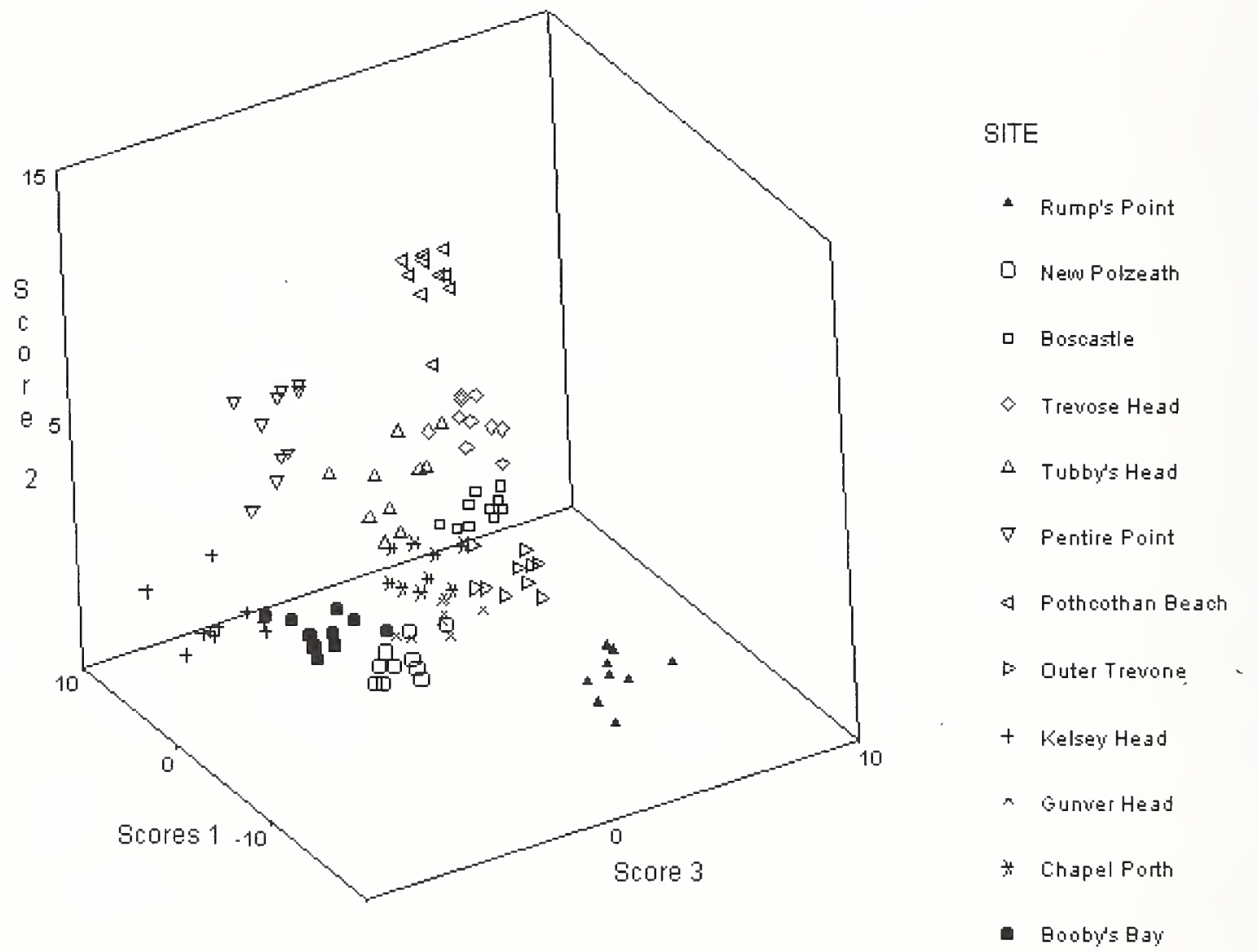


FIGURE 9. Discriminant analysis of individuals from N. Cornwall of *L. britannicum* subsp. *britannicum* A. populations (Key to code in map 1 – Chapel Porth, 2 – Tubby's Head, 3 –Kelsey Head, 4 – Pentire Point, 5 – Porthcothan Beach, 6 – Booby's Bay, 7 – Trevose Head, 8 – Gunver Head, 9 – Outer Trevone, 10 – New Polzeath, 11 – Rumps Point, 12 – Boscastle).

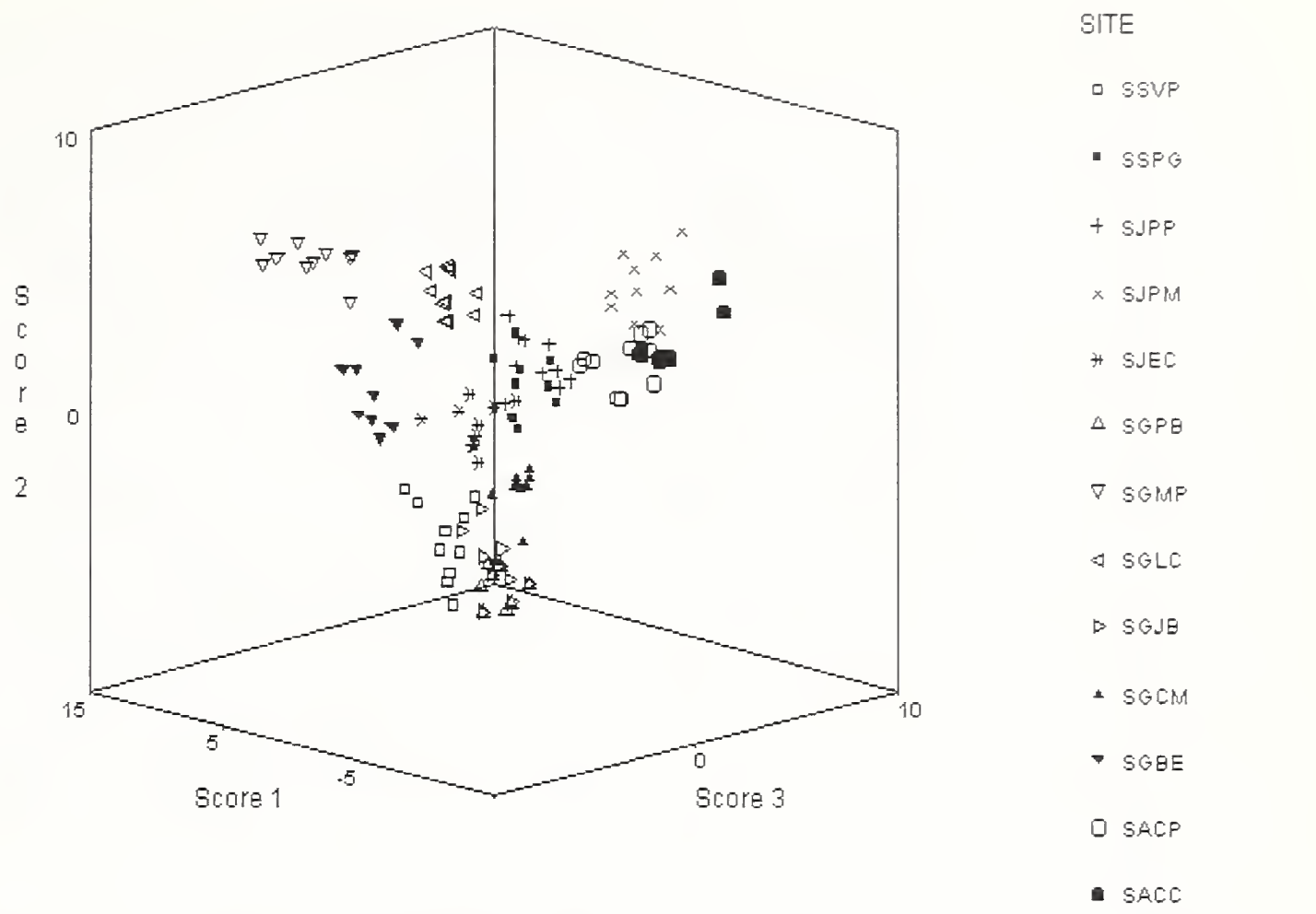


FIGURE 10. Discriminant analysis of individuals from the Channel Islands: Sark, SSVP – Venus' Pool, SSPG – Port Gorey; Jersey SJPP – Plémont Point, SJPM – Point La Moye, SJEC – Elizabeth Castle; Guernsey, SGPB – Petit Bot, SGMP – La Moye Point, SGLC – La Corbière, SGJB – Jerbourg, SGCM – Creux Mahie, SGBE – Belle Elizabeth; Alderney, SACP – Cachalière Point, SACC – Castle de Clonque.

TABLE 2. MISALLOCATED INDIVIDUALS IN DISCRIMINANT ANALYSES BETWEEN THE FOUR WIDE-SPREAD SPECIES OF *L. BINERVOSUM* AGGREGATE INCLUDING EITHER INDIVIDUALS FOR WHICH BOTH FLORAL AND SCAPE AND LEAF CHARACTERS HAD BEEN MEASURED OR, FOR A GREATER NUMBER OF INDIVIDUALS, THOSE IN WHICH FLORAL CHARACTERS WERE MEASURED.

			L. binervosum	*L. procerum*	*L. britannicum*	*L. recurvum*
			Discriminant analysis utilizing all available characters			
		No. of individuals	180	530	260	67
L. binervosum	Discriminant analysis utilizing floral characters only	320		6	4	0
				0.7%	0.7%	0.0%
L. procerum		990	78		36	1
			6.0%		2.9%	0.1%
L. britannicum		490	62	260		0
			7.7%	17.6%		0.0%
L. recurvum		330	34	63	30	
			5.2%	4.8%	3.7%	

TAXONOMIC TREATMENT

This analysis supports the conclusions of the original 1980s analysis that utilised population means as OTUs; there is a pattern of variation that is correlated to geographical distribution that can be apportioned hierarchically to species and infra-specific taxa. It was this hierarchical pattern that was translated into a classification.

However the analysis of individuals emphasises the closeness of species. Either populations are more mixed than previously suspected or, and this is more likely, the normal range of expression of some taxa overlaps with others. In the latter case in order to correctly name an individual it is necessary to examine the whole range of variation in a population.

The two morphologically closest widespread species are *L. procerum* and *L. britannicum*. Neighbouring populations of both these species are found in western Britain and in places it is difficult to discriminate between them. At the least the analysis reported here provides support for the combination of these species perhaps with a relegation to the rank of subspecies. The other widespread species are more distinct but it does require a very wide range of characters to be scored to ensure correct identification. *L. recurvum* is clearly evolutionarily distinct; it is triploid compared to the tetraploid status of the other taxa, but in practise it may be confused in the field with small stature *L .britannicum*. The key character of the roughness of the scape has proved rather variable in nature and difficult to apply if the alternatives (rough and smooth) are not available for comparison. Nevertheless its phylogenetic distinctness is supported by genetic data from an analysis of AFLPs. *L. binervosum* can be more confidently identified though this is largely because of it is mainly allopatric distribution in SE England to the other species in western Britain. However in Sussex and S. Devon there are two populations that disturb this simplicity. A population from Lannacombe in S. Devon has the long spike and distant spikelets of *L. binervosum* and was described as *L. binervosum* subsp. *mutatum* but it has other features such as leaf shape that relate it to the neighbouring populations of *L. procerum*. In addition at Rottingdene (v.c. 14) in Sussex there is a possibly introduced population of *L. procerum* on the margin of the range of *L. binervosum* and very isolated from the nearest *L. procerum* populations in S. Devon.

The analysis reported here indicates that perhaps *L. binervosum* agg. has been over-taxonomised, with taxa recognised at too high a rank, not an unusual situation in agamospermous groups where it has been practise to describe myriad microspecies. However there is a more fundamental outcome of these results that may undercut the whole classification. The results of an analysis of individuals reveals much greater variation within populations than previously noted and places in doubt the narrow endemic species that have previously been described. Within the widespread species even geographically adjacent populations of the same taxonomic variety can be discriminated by multivariate statistical techniques. Previously some populations that had a readily utilisable character that enabled their identification, such as the smaller floral parts of *L. britannicum* var. *kelseyanum* and the lax scapes of *L. binervosum* subsp. *sarniense* var. *aurigniense*, were described, but the analysis reported here shows that these are no more distinct and no more deserving of taxonomic recognition than other populations.

In this context the narrowly endemic species, two of which have been recognised for decades, *L. transwallianum* (Pugsley 1924) and *L. paradoxum* (Pugsley 1931) and *L. parvum*, *L. dodartiforme* and *L. loganicum* described in the 1980s (Ingrouille & Stace 1986), may be only variant populations with readily utilisable identifying characters. In contrast it was the discovery of a shared triploid condition in several other populations,

especially in W. Ireland, that expanded the narrow concept of *L. recurvum* of Salmon (1903) perhaps making this widespread species rather difficult to comprehend because of the morphological variation it then contained.

The morphometric analysis of populations (Ingrouille & Stace 1985) and of individuals reported here is broadly supported by the results of the analysis of AFLPs but the genetic distance varies quite considerably from the morphometric distance. The AFLP analysis indicates *L. parvum* and *L. transwallianum* are genetically close to *L. procerum/britannicum*; *L. paradoxum* and *L. loganicum* are close to *L. binervosum sensu stricto*; and *L. dodartiforme* is close to *L. binervosum* agg. from mainland Europe.

The greatest genetic distances are found within *L. binervosum sensu stricto*, in which the subspecies are genetically more distant from each other than many other species are in the aggregate despite their morphological closeness. In particular *L. binervosum* subsp. *anglicum* is as genetically distant from other *L. binervosum* subspecies as is *L. recurvum*, though it is morphologically close to *L. binervosum* subsp. *binervosum*.

Leaving aside the question of which should have primacy in determining taxonomic delimitation and rank, genetic distance or morphological distance, this analysis indicates strongly that the formal recognition of many specific and infra-specific taxa in *L. binervosum* agg. in the British Isles may be of limited value. It may be more accurate and useful to adopt a more informal naming system, incorporating the sample site, a kind of polynomial, such as 'the *L. binervosum* variant with a lax spike from Lannacombe Beach (v.c. 3), S. Devon' rather than *L. binervosum* subsp. *mutatum*. This may seem a more cumbersome approach but it is less misleading than trying to lever a complex nexus of relationships into the straitjacket of a hierarchical classification.

THE EVOLUTION OF THE *L. BINERVOSUM* AGGREGATE IN THE BRITISH ISLES

These results pose a different kind of problem. *L. binervosum* is an obligate agamosperm. This is indicated by the male sterility of plants (lack of pollen or poorly stained and distorted pollen) and by the presence of a pollen and stigma combination (A/Cob) that prevents self–pollination (Ingrouille 1982). The A pollen/Cob stigma is half of the dimorphic self-incompatibility system found in sexual species of *Limonium* and requires the alternative B pollen/papillate stigma combination for successful pollination (Baker 1966), but the latter is entirely absent in the British Isles.

As a consequence the evolution of the *L. binervosum* aggregate has been entirely asexual. Nevertheless a complex hierarchical pattern of variation has arisen and the geographical correlation of this pattern indicates strongly that it has occurred *in situ* within the last 10,000 years since the end of the last glacial period. How has it evolved so rapidly? Where is the source of variation that has provided the opportunity of evolutionary divergence? This has been a significant problem in trying to understand the evolution of this group.

In part the results reported here, that much more variation is present within populations than was previously suspected, goes some of the way to answering this conundrum. In fact variability within taxa has been previously reported, for example in pollen morphology and flower colour, but this was previously thought not to be significant. In a small number of cases, where it included a clearly marked variant and it was so clear that it could not be ignored, it led to the description of varieties within the same population. For example at St David's Head (v.c. 45) and in its vicinity three different variants, differing in spike morphol-

ogy, were described (*L. paradoxum* var. *paradoxum* and var. *mutabile* and L. *procerum* var. *paramedium*). Multiple variants of *L. recurvum* from the isle of Portland were described. Now it is clear that these cases are just examples of a more widespread phenomenon in *L. binervosum* aggregate. Here is the material that natural selection can act upon.

However where is the source of this variation? Genetic mutation may have played a large part. Released from a normal meiosis by agamospermy the rate of mutation may have been enhanced. Chromosomal variation provides evidence of one kind of mutation with differences in ploidy level (triploid and tetraploid) and aneuploidy between plants and populations, but most tetraploids have 2n = 35 (4x-1) and only *L. paradoxum* has 2n = 33 (4x-3).

However, mutation rates recorded from a range of plants are generally too low (Gustafsson, 1951). A direct indication of rates of mutation in *L. binervosum* agg. is provided from the results of the study of AFLPs (Cowan *et al.* 1998). AFLPs are polymorphic markers in the most rapidly evolving part of the genome, in the repetitive DNA, but the level of AFLP variation between *L. binervosum* taxa is low in comparison to sexual species. It therefore seems unlikely that mutation rates are enhanced in *L. binervosum* aggregate.

Especially in agamospermous taxa hybridisation has been invoked as a potential source of variation. The complex pattern of variation observed is seen merely as the consequence of residual sexuality and occasional sexual crossing, with asexual reproduction merely multiplying and fixing new hybrid variants. Hybridisation has been suggested in *Limonium* among Mediterranean taxa (Erben 1978, 1979) and in the origin of sister species differing in pollen/stigma morph in the *L. ovalifolium/L. auriculae-ursifolium* group (Ingrouille 1985). However the complete lack of sexuality in *L. binervosum* agg. in the British Isles makes this hypothesis very unlikely, at least here.

An alternative source of variation would be if the differences between taxa are not genetic but epigenetic, arising from differences in patterns of gene expression in development. For example different patterns of methylation of the genome modulate gene expression and are potentially heritable. There is considerable evidence for this Non-Mendelian/Lamarckian possibility (Jablonka & Lamb 1998). Data from an analysis of AFLP variation within and between *L. binervosum* aggregate populations (R. Cowan personal communication) indicates extremely low levels of variation, even between the most distinct morphological microspecies. The level of variation is an order of magnitude different from that detected in other agamospermous groups where residual sexuality is present (*Taraxacum*, *Sorbus*) (Hughes & Richards 1989, Asker 1980) and provides strong support that morphological differences detected are epigenetic.

It is becoming more clear that the relationship between genotype and phenotype is not direct but is modulated by a complex nexus of relationship between gene and environment in the course of development. An analogy is with the BIOS (basic input/output system) of computer systems that provides the link between the hardware and the software (Fig. 11). Some of the BIOS is burned or flashed into a ROM chip that is both non-volatile and read-only, some of the BIOS is included on ROM chips installed on adapter cards, and some of the BIOS are additional drivers loaded when the system boots up.

Potentially the BIOS of *L. binervosum* plants can evolve much more rapidly. Plants can look more similar than their genetic distance indicates or more different than their genetic closeness indicates. How significant are developmental differences (the 'BIOS') in the evolution of organisms? It may allow organisms to rapidly adapt to different circumstances and it may alter the rate of genetic change by shielding genetic variation from selection. There is direct evidence that some variation detected is potentially of ecological significance,

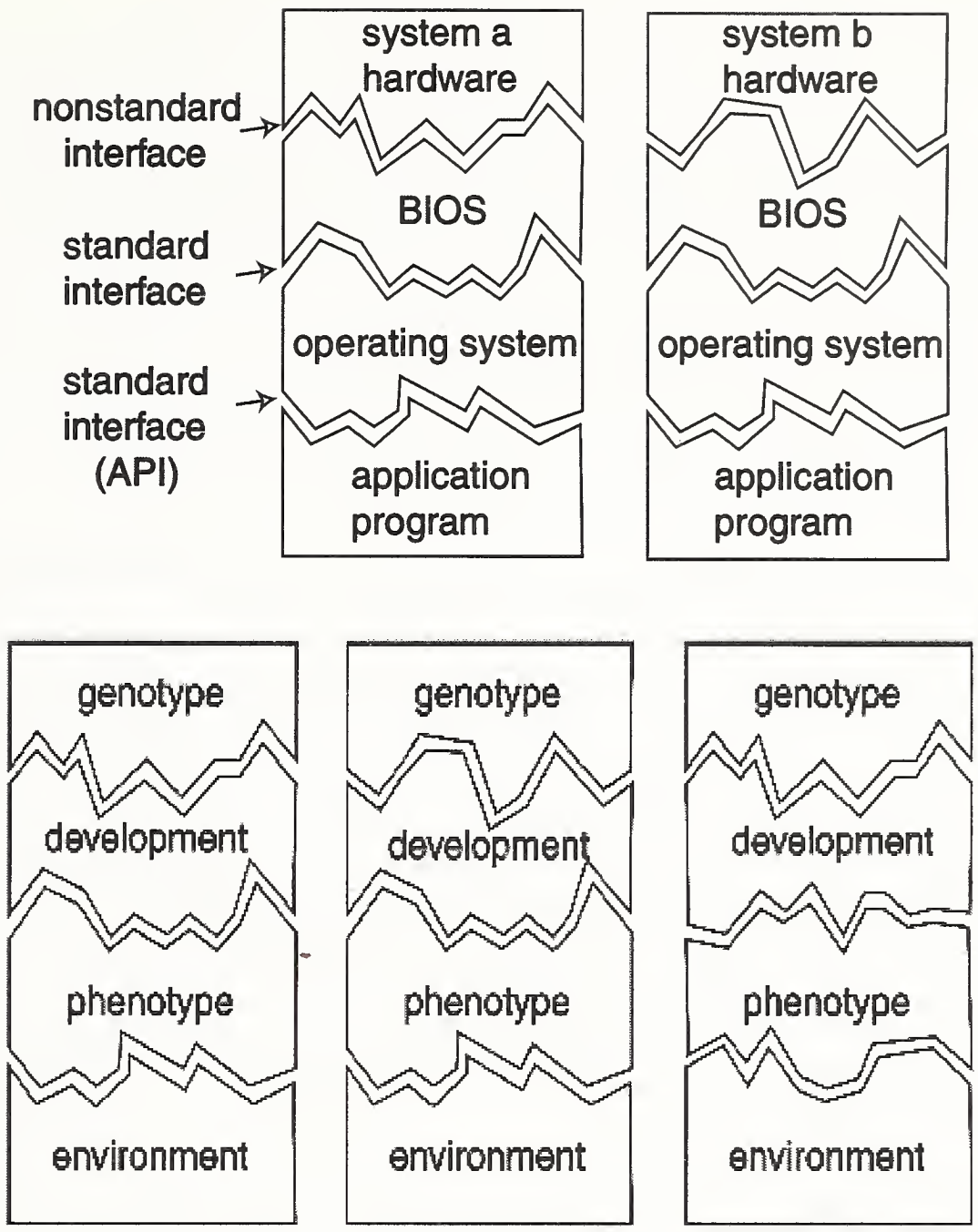


FIGURE 11. An analogy for the relationship between genotype and phenotype from the operating systems of personal computers: A. the BIOSIS mediates the link between the computer hardware and software allowing different hardwares to run the same software or the same software to run different kinds of software; B. the biological BIOSIS, the developmental nexus, permits plants with different genotypes to survive in similar circumstances, or with the same genotype to survive in different circumstances.

i.e. has been selected (Ingrouille 1982). When grown together variants differ in time of flowering; those with a natural western distribution flower earlier than those with an eastern distribution. Variants differ in their rate of seed germination. Variants normally found above the margins of salt-marshes are more sensitive to sea-water than those normally found growing on cliffs.

If developmental differences are inherited in a significant degree then neither genetic distance nor morphological distance (as a measure of phenotypic distance) is an entirely reliable indicator of species limits, bringing us back to the question of what is more important in classification at species rank and below - the phenotype or the genotype? The phenotype is more fully and more readily measurable as a totality – it also connects the plant to its environment – whereas only a miniscule part of the genotype can be measured and the part that is measured is likely to have no relevance to the plant in its environment. However the genetic evidence provides a strong break on the impulse to over-taxonomise a variable species group, as has happened in the *Limonium* in general and in the *L. binervosum* aggregate in particular.

REFERENCES

ASKER, S. (1980). Gametophytic apomixes: elements and genetic regulation. *Hereditas* **93**: 277–293.

BAKER, H.G. (1966). The evolution, functioning and breakdown of heteromorphic incompatibility systems I. The Plumbaginaceae. *Evolution* **20**: 349–368.

COWAN, R., INGROUILLE, M.J. & DOLORES LLEDO, M. (1998). The taxonomic treatment of agamosperms in the genus *Limonium* Mill., (Plumbaginaceae). *Folia Geobotanica* **33**: 353–366.

ERBEN, M. (1978). Die Gattung *Limonium* im Südwestmediterranen Raum. *Mitteilungen Botanische Staatsammlung München* **14**: 361–631.

ERBEN, M. (1979). Karyotype differentiation and its consequences in Mediterranean "Limonium". *Webbia* **34**: 409–417.

GUSTAFFSON, Å. (1951). Induction of changes in genes and chromosomes. II. Mutations, environment and evolution. *Cold Spring Harbour Symposium of Quantitative Biology* **16**: 263–281.

HUGHES, J. & RICHARDS A.J. (1989). Isozymes, and the status of *Taraxacum* (ASTERACEAE) Agamospecies. *Botanical Journal of the Linnean Society* **99(4)**: 365–376.

INGROUILLE, M.J. (1982). *The colony structure and taxonomic characterisation of apomictic Limonium (Plumbaginaceae) in the British Isles*. PhD thesis, University of Leicester.

INGROUILLE, M.J. (1985). The *Limonium auriculae-ursifolium* (Pourret) Druce group (Plumbaginaceae) in the Channel Isles. *Watsonia* **15**: 221–229.

INGROUILLE, M.J. & STACE, C.A. (1985). Pattern of variation of agamospermous *Limonium* (Plumbaginaceae) in the British Isles. *Nordic Journal of Botany* **5**: 113–125.

INGROUILLE, M.J. & STACE, C.A. (1986). The *Limonium binervosum* aggregate (Plumbaginaceae) in the British Isles. *Botanical Journal of the Linnean Society* **92**: 177–217.

JABLONKA, E. & LAMB, M. (1998). Epigenetic inheritance in evolution. *Journal of Evolutionary Biology* **11**: 159–183.

PUGSLEY, H.W. (1924). A new *Statice* in Britain. *Journal of Botany* **24**: 129–134.

PUGSLEY, H.W. (1931). A further new *Limonium* in Britain. *Journal of Botany* **69**: 44–47.

SALMON, C.E. (1903). Notes on *Limonium*. *Journal of Botany*, **41**: 64–74.

STACE, C.A. (1997). *New Flora of the British Isles* (second edition). Cambridge University Press, Cambridge.

Current taxonomic research on the British & European flora
Bailey, J. & Ellis, R.G. (eds) 89–110 (2006), BSBI. London.

How many orchid species are currently native to the British Isles?

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ABSTRACT

The British Isles probably host the most intensively studied flora in the world, and within that flora the Orchidaceae has long been the most intensively studied family. Nonetheless, molecular phylogenetic studies performed only during the last decade have revolutionised our understanding of species relationships among European orchids, eliminating former monotypic genera such as *Aceras*, *Coeloglossum* and *Hammarbya*, apportioning many former *Orchis* species to expanded concepts of *Anacamptis* and *Neotinea*, and combining '*Listera*' into *Neottia*. Emphasis has now switched from species comparison to species delimitation, integrating morphometric approaches with population genetic techniques via simultaneous ordinations. Early results suggest that several distinct speciation mechanisms operate within the British and Irish orchid flora, and challenge the validity of several 'Schedule 8' species. No meaningful differences exist between British *Dactylorhiza* '*lapponica*' and *D.* '*traunsteineri*', and neither represents the same allopolyploid speciation event as *D. traunsteineri* from the type locality in Austria. Also, contrary to the recent *Atlas of the British & Irish flora*, *D. majalis s.s.* does not occur in the British Isles. By contrast, three habitat 'races' within *Gymnadenia* merit species status. *Epipactis* '*youngiana*' is not reliably distinct from *E. helleborine*, whereas the autogamous *E. leptochila* and *E. dunensis* both warrant species status, alongside *E. sancta* recently described from Lindisfarne. Controversial taxa are either widely recognised but lack biological cohesion (Emperor's New Clothes species), rarely if ever recognised but possess biological cohesion (Cinderella species, including Robinson Crusoe species recently diverged on islands such as *Dactylorhiza ebudensis*), or are migrating northward, presumably in response to climate change (Bleriot species, such as *Serapias parviflora*). Recent arrivals by origination or migration are partly negated by extirpation of longer established species, notably *Spiranthes aestivalis* and arguably *Epipogium aphyllum*. Present evidence suggests that the orchid flora of the British Isles (excluding the biogeographically French Channel Islands) currently consists of 52 species in 20 genera; these taxa are herein reclassified in anticipation of the third edition of Stace's *Flora.*

Keywords: classification, critical groups, DNA, Europe, morphometrics, Orchidaceae, ordination, phylogenetics, population genetics, speciation.

INTRODUCTION

I have chosen to mark the notable occasion of Clive Stace's retirement on my home turf, by preparing a benchmark overview of recent insights gained mainly from DNA-based studies into the biology, systematics and evolution of the British and Irish orchid flora. This text connects with my Presidential address to the UK Hardy Orchid Society (Bateman 2004). Here, I will focus especially on how best to integrate a wide range of morphological and molecular data in order to circumscribe species and infraspecific taxa, highlighting the most species-rich orchid genera in the British Isles, *Epipactis* and *Dactylorhiza.*

PERSPECTIVES ON EVOLUTION

Evolution can be viewed in two ways. We are most familiar with seeing it viewed laterally, morphological divergence among species being plotted against time as the vertical axis to generate the familiar tree motif (Fig. 1, bottom). However, we can also usefully view the present-day products of evolution from above and at 'higher magnification', seeking morphological gaps among sets of individuals representing particular populations (Fig. 1, top); these gaps should in theory reflect barriers to gene exchange between species. Seen from above, species are less frequently visualised as a tree but more often as clusters of points on two-dimensional 'ordination' plots; these resemble the simple graphs of the schoolroom but are based on more sophisticated statistical methods that summarise many more variables.

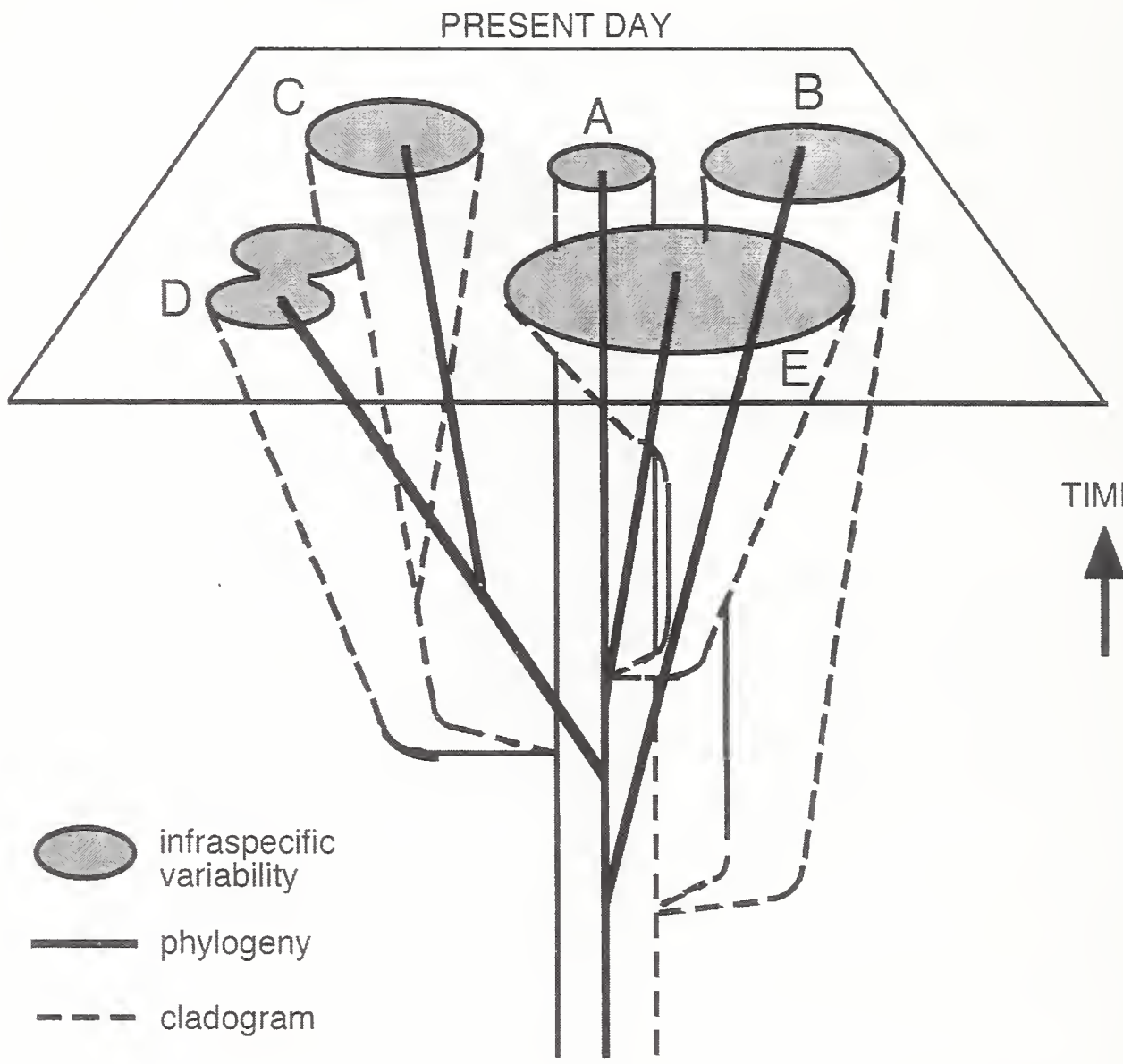

FIGURE 1. Comparison of perspectives of evolution as viewed laterally through time, dominantly represented by the tree motif, and viewed perpendicular to the present time-plane, when taxa are best represented as clusters of individuals separated by discontinuities

Evolution inevitably involves changes in the appearance or biological 'behaviour' of a species that are caused by one or more genetic modifications. Thus, in order to understand evolution, we need to describe both the morphology *and* the molecular genetics of each organism, and then compare the patterns revealed by these complementary sets of techniques. When comparing species by generating evolutionary trees (phylogenetics) we tend to represent each species with a single supposedly typical individual (an approach to sampling that can be termed typology, analogous to the type specimens of traditional taxonomy: e.g. Stace 1989). Consequently, the morphological data most appropriately

employed in such studies reflect discrete characters that are either present or absent, while corresponding genetic data are sequences of bases from particular regions of the DNA of the plants (both reviewed in detail by Bateman 1999, 2001).

However, when attempting to circumscribe species, we need information from much larger numbers of individuals sampled from across the geographical range of each suspected species. Morphological data are diverse, including counts and measurements of particular structures; similarly, molecular genetic approaches are more diverse, including not only sequences but also techniques that dissect the DNA into comparable fragments and then measure with great precision their contrasting sizes. Such population-level studies are more labour-intensive, and because of the necessity to sample extensively within species they cannot encompass nearly as many species as tree-building approaches. In practice, each of these approaches feeds relevant information into the other; indeed, this feedback is reflected in most of the case-studies described below.

We are fortunate that the orchids native to the British Isles have in the last decade probably been analysed more extensively using these techniques than any other plant family anywhere else in the world. This collective success reflects strong research collaborations between the three main plant systematics laboratories in Britain (Royal Botanic Gardens Kew, Royal Botanic Garden Edinburgh, and latterly Natural History Museum), working together with other universities located further afield (e.g. Lund, Naples, Estonia, Beijing). The initiative was also driven in part by materials supplied to the research team by 'amateur' orchidologists, particularly members of the UK Hardy Orchid Society and the Botanical Society of the British Isles. This review unashamedly focuses on these UK-based successes.

MOLECULAR AND MORPHOLOGICAL PHYLOGENIES: SPECIES COMPARISON

The results of studies constructing evolutionary trees of the tribes Orchideae (*Orchis*, *Dactylorhiza* and their relatives) and Neottieae (*Epipactis*, *Neottia* and their relatives), revolving around Kew's comprehensive *Genera Orchidacearum* project, have emerged gradually over the last seven years (e.g. Bateman *et al.* 2003, 2004). The original sequence data derived from the ITS region of the nuclear genes, which is inherited from both parents of a plant, have since been supplemented with sequences from two regions of the plastids (mainly chloroplasts), which are inherited only from the mother. The two different clusters of DNA (termed genomes) in the nucleus and plastids are under different constraints and so can inform us about different aspects of the evolution and genealogy of the plants being analysed. The resulting phylogenies are considered more reliable when these contrasting sources of sequence data are broadly in accord.

Comparison of the nuclear and plastid results confirms the original ITS-based interpretations (Fig. 2) in all cases. *'Aceras' anthropophora* is in fact a true 'anthropomorphic' *Orchis*. In contrast, '*Orchis*' *ustulata* has no close relationship with *Orchis purpurea*, being part of the group epitomised by *Neotinea maculata*, and '*Orchis*' *morio* has no close relationship with *Orchis mascula*, being nested between *Anacamptis pyramidalis* and *A.* ('*Orchis*') *laxiflora*. *'Coeloglossum' viridis* is actually a near-basal diploid *Dactylorhiza*. More recent discoveries include the fact that *Neottia nidus-avis* is closely related to, and may have evolved from, '*Listera*' *ovata*; species formerly in the genus *Listera* should therefore be transferred to *Neottia* (Bateman *et al.* 2005). Within Tribe Malaxideae, a case could be made for sinking *Hammarbya paludosa* into *Liparis*, but current evidence suggests that it is

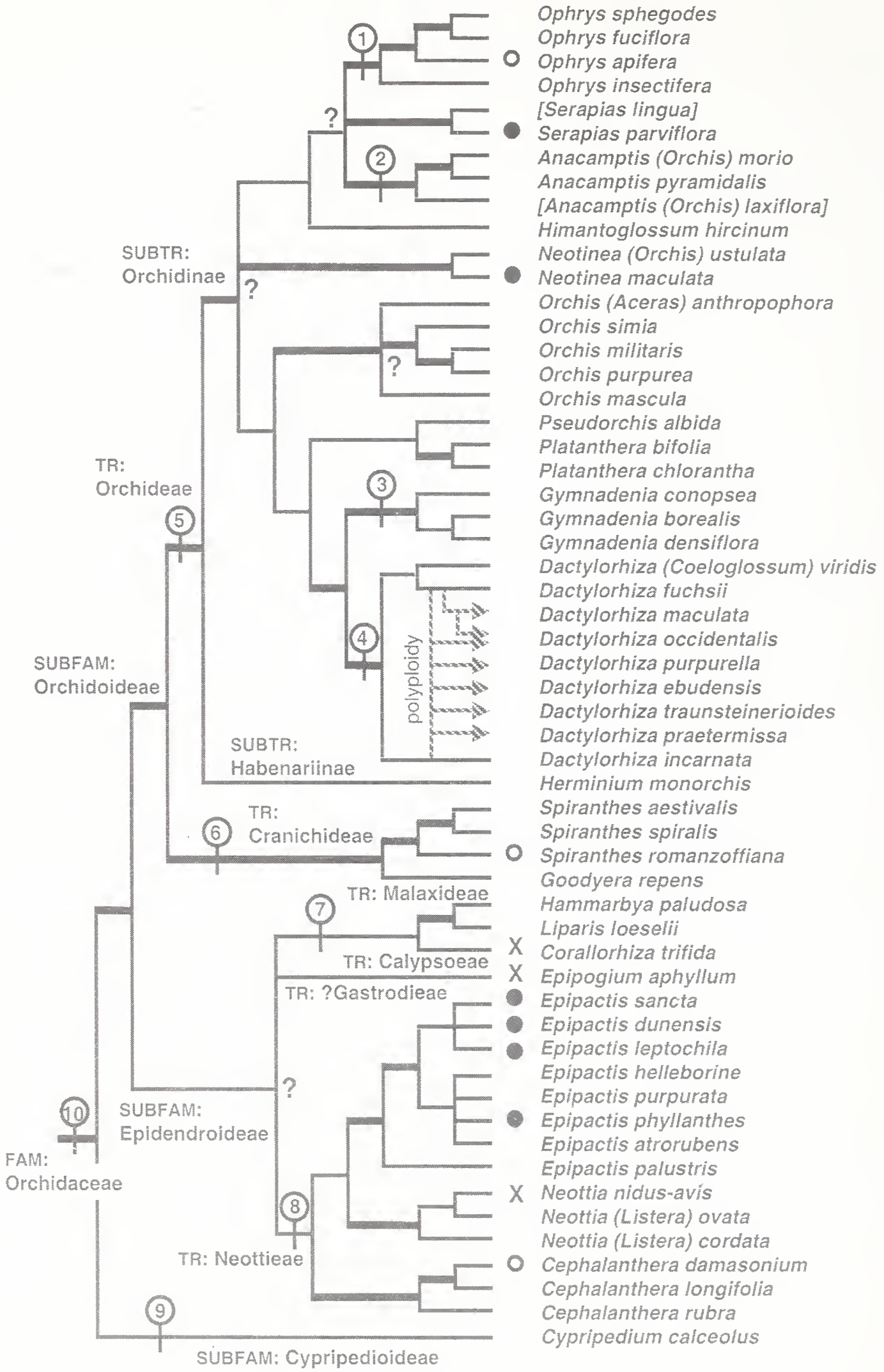

FIGURE 2. Grafted aggregate phylogeny of all British and Irish orchid species, based on several more focused analyses. Thick branches denote the more reliable relationships. Crosses indicate obligate myco-heterotrophs; spots indicate obligate autogams, circles indicate facultative autogams.

Circled numbers indicate sources of component cladograms: 1 Soliva *et al.* 2001, 2 Bateman & Hollingsworth 2004, 5 Bateman *et al.* 2003, 3 Bateman *et al.* in prep., 4 Pillon *et al.* in press., 6 Salazar *et al.* 2003, 7 Salazar *et al.* in prep., 8 Bateman *et al.* 2005, 9 Cox *et al.* 1997; 10 Dressler 1993, Cameron *et al.* 1999, Freudenstein & Rasmussen 1999.

sufficiently isolated and distinct to warrant maintenance as a monotypic genus (Salazar, Chase & Cribb in prep.; contra Bateman 2004).

The almost complete coverage of species achieved now greatly facilitates further systematics research, both within and outwith the British Isles. Firstly, it is easy to place phylogenetically the few remaining unplaced Eurasian species. For example, analysis of the contentious Balkan taxon '*Pseudorchis*' *frivaldii* clearly demonstrates that this species is nested within *Gymnadenia*, close to (but apparently not sister to) the former genus *Nigritella* (Bateman, Rudall & James 2006). This evolutionary tree shows that floral reduction has occurred three times in Continental Europe within *Gymnadenia* (it has already been demonstrated to have occurred separately in '*Nigritella*' and *G. odoratissima*). Secondly, the evolutionary tree provides a robust framework for re-examining records of natural hybridisation (cf. Stace 1975). For example, applying molecular phylogenetic techniques to a suspected hybrid from Mallorca new to science, between *Anacamptis* (formerly *Orchis*) *fragrans* and *A. robusta*, demonstrated not only that its parentage had been correctly identified but also that *A. fragrans* was its mother and had passed on to the adjacent hybrid more of its morphological characteristics than had its father, which was located at least 100 m distant (Bateman & Hollingsworth 2004). Such techniques are now being applied to hybrids in more challenging genera such as *Dactylorhiza* and *Ophrys*, as well as explaining degrees of historical introgression among the 'anthropomorphic' *Orchis* species *O. anthropophora*, O. *simia*, *O. militaris* and *O. purpurea* (Qamaruz-Zaman 2000; Bateman in press; Fay *et al.* in prep.), three of which are now awarded Schedule 8 conservation status.

MORPHOMETRICS AND POPULATION GENETICS: SPECIES DELIMITATION

Armed with both morphometric and population genetic tools, we can in theory re-examine the status of *all* of the supposed orchid species presently regarded as native to the British Isles.

EPIPACTIS

Several techniques have already been applied to one of the most troublesome genera, *Epipactis* (Squirrell *et al.* 2001, 2002; Bateman *et al.* 2005). Sampling across Europe indicates at least a dozen independent and randomly-distributed origins of self-pollinated lineages from within the cross-pollinated *E. helleborine* complex. Each self-pollinated line is genetically distinct and, because of being self-pollinated, possesses exceptionally low genetic diversity. However, this supposed genetic weakness has not prevented species such as *E. phyllanthes* and *E. leptochila* from becoming widespread across western Europe. *Epipactis dunensis* is a British endemic that is genetically distinct from the more widespread *E. leptochila*; moreover, the single small population of *Epipactis* on the dunes of Lindisfarne in northeast England also appears sufficiently distinct genetically to provide some support for Delforge's molecularly-inspired decision to describe it as a new species, *E. sancta* (Colour Plate 1f) (Delforge & Gévaudan 2002).

However, another supposed British endemic *Epipactis*, *E.* '*youngiana*', does not pass muster. First described in 1982 and soon elevated to Schedule 8, it was subsequently explored using protein-based allozyme analysis (Harris & Abbott 1997). Results indicated that, in any one population, *E. youngiana* could not be separated effectively from the inevitably co-existing populations of *E. helleborine*; in other words, individuals assigned to

E. youngiana were merely plants of *E. helleborine* with unusually small rostella that consequently were unable to guard against self-pollination. This observation has since been confirmed using more sophisticated sequence-based population genetic techniques (Hollingsworth *et al.* 2006).

Current evidence suggest two main causes of diversification of British *Epipactis*: mutationally-driven rostellum loss, and expansion into subtly different habitats and soils that may in part reflect switches of mycorrhizal partners (Bidartondo *et al.* 2004; Bidartondo, Reid & Bateman unpubl.).

DACTYLORHIZA

An even broader panoply of molecular techniques has been applied to the most taxonomically troublesome of all of Britain's orchid genera, *Dactylorhiza*. Many authors ascribe the morphological complexity in the genus to 'hybridisation'. There is some truth in this statement, but the most crucial mode of hybridisation in *Dactylorhiza* is when two morphologically and genetically contrasting diploid species (most commonly *D. incarnata s.l.* and *D. fuchsii s.l.*) simultaneously hybridise and double their chromosome number in the progeny. This process, termed allopolyploidy (e.g. Stace 1975), immediately confers on the progeny fertility and at least partial genetic isolation from their parents. Key questions to address are therefore how frequently this process results in successful establishment of the resulting stabilised hybrid lineages, and which species is the mother and which the father in each case.

By comparing results from a wide range of different analytical techniques (Table 1), it has become clear that members of the *D. incarnata s.l.* and *D. fuchsii s.l.* groups have combined repeatedly to generate large numbers of subtly distinct 'species' (Hedrén *et al.* 2001; Pillon *et al.* subm.). In Greece and Asia Minor, *D. euxina* replaces *D. incarnata* and *D. saccifera* replaces *D. fuchsii* as parents of the allopolyploids (Hedrén 2001, 2003), whereas in Ireland and northwest Scotland *D. maculata* sometimes replaces *D. fuchsii*. A similar situation characterises north-west Russia, where *D. baltica* replaces the morphologically similar northern British/Nordic *D. purpurella* as the dominant allotetraploid (Shipunov & Bateman 2005). In this case, the lip shape (as summarised via landmark analysis) of an individual correlates well with species identity. The strong first axis separates *D. fuchsii* from the less deeply-lobed *D. incarnata* and *D. maculata*, and places *D. baltica* (the allotetraploid derivative of hybridisation between *D. fuchsii* and *D. incarnata*) between its two parental diploids. Moreover, lip shape can successfully be related to the proportion of alleles of the Internal Transcribed Spacer (ITS) nuclear ribosomal DNA region estimated to have been inherited from each parental diploid by *D. baltica* (Fig. 3). This technique should now be applied to British and Irish populations.

As with *Epipactis*, some peripheral populations of *Dactylorhiza* have proved to be both recently evolved and genetically unique. A good example is *D. ebudensis* (Colour Plate 1b), found only on the Hebridean island of North Uist, which is unusual among British allotetraploid dactylorchids in having *D. incarnata* rather than *D. fuchsii* as its mother (it appears to be *D. incarnata coccinea* × *D. fuchsii hebridensis*). Also, some allopolyploid species have proved to have multiple origins; for example, *D. traunsteineri* has separate origins in the Alps (where the type locality is situated), Scandinavia and the British Isles (Hedrén *et al.*

TABLE 1. DIPLOID (BOLDFACE) AND TETRAPLOID SPECIES OF *DACTYLORHIZA* FROM THE BRITISH ISLES GROUPED ACCORDING TO SEVEN DIFFERENT KINDS OF ANALYSIS. Asterisked studies were based largely on Continental material.

Morphometrics	**Allozymes**	**AFLPs**	**Nuclear ITS sequences**	**Plastid sequences**	**Plastid microsatellites**	**Plastid PCR-RFLPs**
Bateman & Denholm 1983 et seq.; Pedersen 1998*	Hedren 1996*, 2001*; Bateman, McLeod, Craig, Hedrén & Ennos unpubl.	Hedrén *et al.* 2001*	Bateman *et al.* 2003; Pillon *et al.* in press	Bateman & Denholm 2003 (*trnL*); Pillon & Chase unpubl. (*rpl16*)	Pillon *et al.* in press.	Devos *et al.* 2004*
incarnata (sev. taxa) ***cruenta***	***incarnata*** (sev. taxa: UK)	***incarnata*** (sev. taxa) ***cruenta***	***incarnata*** (sev. taxa) ***cruenta*** *ebudensis* *purpurella* *cambrensis*	***incarnata***	***incarnata*** (sev. taxa) ***cruenta*** *ebudensis*	***incarnata***
	incarnata (Continent) ***cruenta*** (UK + Continent)					
viridis	***viridis***	***viridis***	***viridis***	***viridis***	***viridis***	
maculata	*maculata*	*maculata*	*maculata* *occidentalis 1*		*maculata* *occidentalis*	*maculata* ***fuchsii 2***
fuchsii (sev. taxa)	***fuchsii***	***fuchsii***		***fuchsii*** *maculata*		***fuchsii 1***
praetermissa *junialis*	*praetermissa* *?junialis*	*praetermissa*	*praetermissa* *junialis* ***fuchsii 1*** (sev. taxa)			*praetermissa*
traunsteinerioides *'lapponica'* *ebudensis*	*traunsteinerioides* *'lapponica'* *ebudensis*	*traunsteinerioides* *'lapponica'* *ebudensis*	*traunsteinerioides* *'lapponica'* *?occidentalis 2* ***fuchsii 2***	[Allotetraploids not analysed]	*traunsteinerioides* *'lapponica'* *praetermissa 1* *?junialis*	
purpurella *?cambrensis*	*purpurella* *cambrensis*	*purpurella* *?cambrensis*			*purpurella* *cambrensis* *?praetermissa 2* ***fuchsii***	
occidentalis						

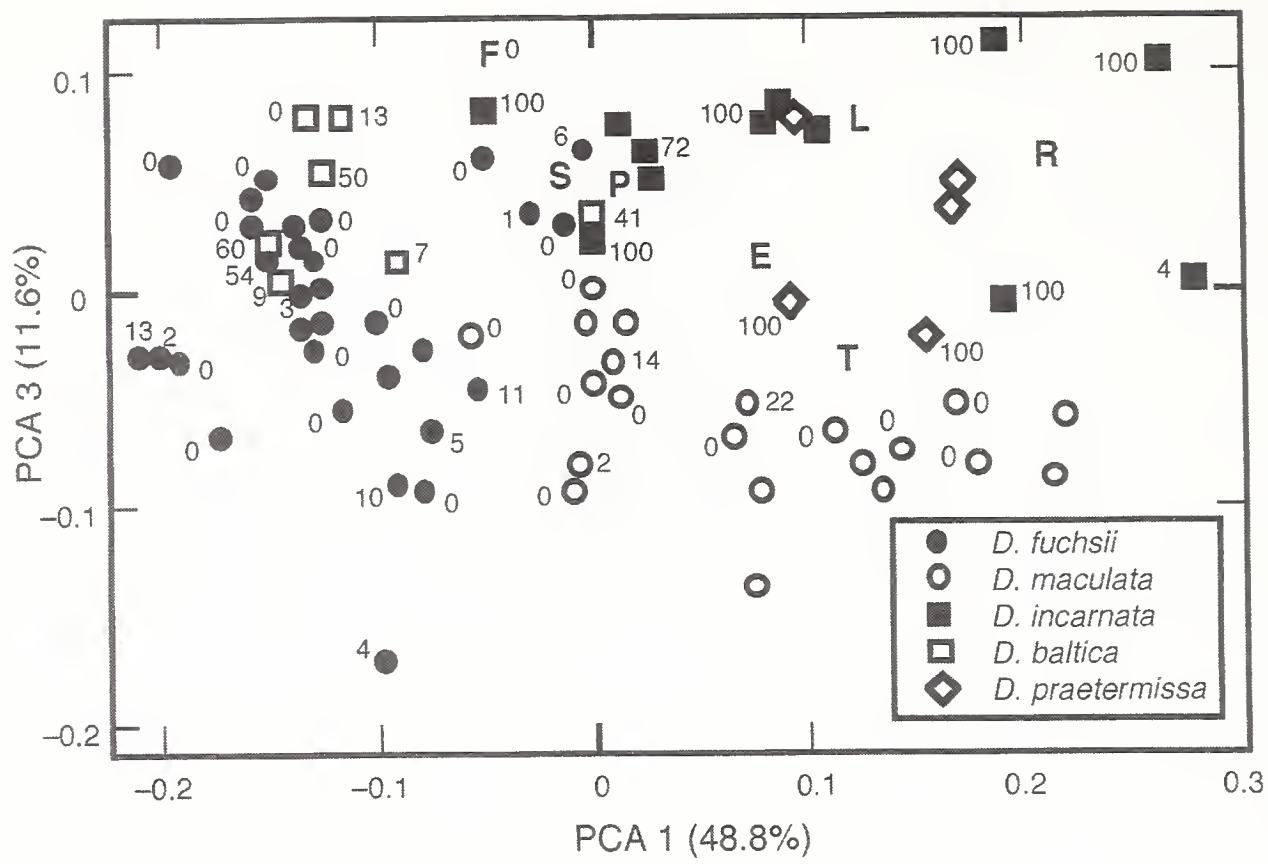

FIGURE 3. Principal components ordination of labellum shapes of 83 individual dactylorchids, most from European Russia, summarised via principal warps analysis of landmark data. Superimposed figures indicate the estimated proportion of ITS alleles in each of 52 individuals analysed genetically that can be sourced to *D. incarnata* rather than to *D. fuchsii* or other closely related diploid species. Letters indicate single placeholders of other comparable species, most from western Europe. Diploids: F = *D. flavescens* (*romana* group), E = *D. euxina* (*incarnata* group); tetraploids of *traunsteineri* group: T = *D. traunsteineri*, L = *D. lapponica*, R = *D. russowii*; tetraploids of other groups: P = *D. purpurella*, S = *D. sphagnicola.* (Derived from Shipunov & Bateman 2005, figs 3, 4.)

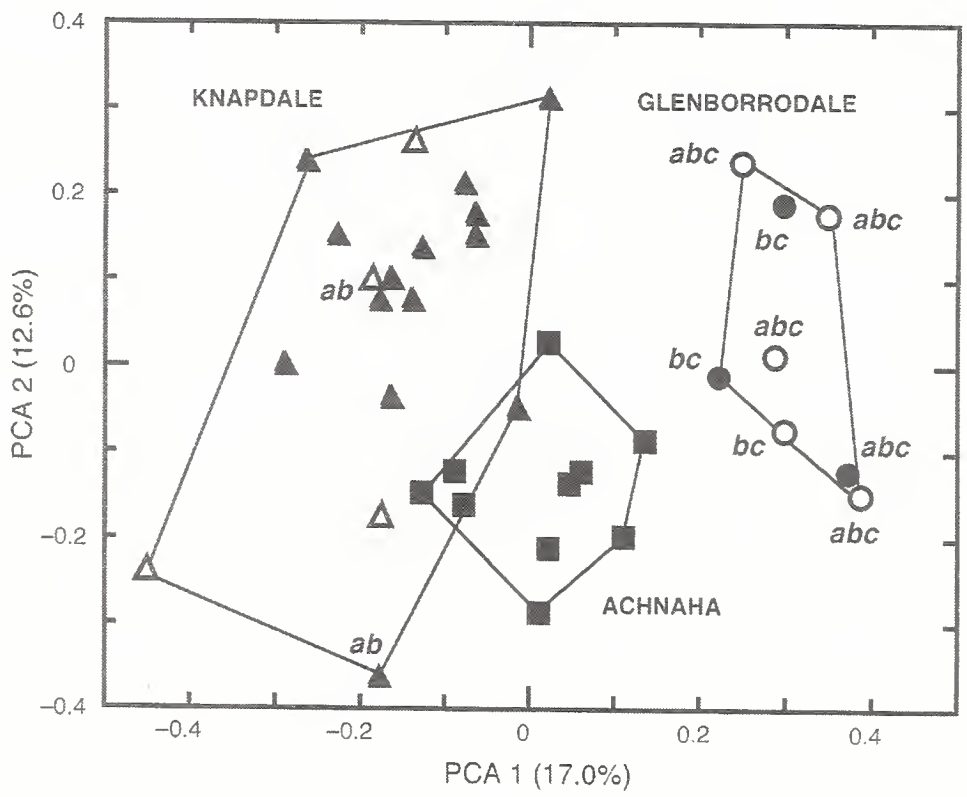

FIGURE 4. Simultaneous principal components ordination of morphometric and allozymic data for 36 individuals from three populations of *Dactylorhiza traunsteineri s.l.* from western Scotland, with superimposed allozyme genotypes for the *6-pgd* locus (individuals without annotation possess the *ac* genotype). Closed symbols indicate that leaf markings are present (i.e. supposed *D. lapponica),* whereas open symbols indicate that leaf markings are absent (i.e. supposed *D. traunsteineri*) (Modified after Bateman 2001, Fig. 7.)

2001; Pillon *et al.* subm.); thus, British populations are more correctly named *D. traunsteinerioides* (Bateman & Denholm 1983).

Moreover, many populations of *D.* '*traunsteineri*' in Scotland are admixed with populations of the Schedule 8 'species' *D. lapponica* (McLeod 1995; Bateman 2001). Figure 4 is an ordination of morphometric data from three populations of the *D. traunsteineri* aggregate from alkaline fens in western Scotland: one population is viewed as pure *D. lapponica* (Achnaha) whereas the other two geographically well-separated populations supposedly contain both *D. lapponica* and *D. traunsteineri* (Glenborrodale and Knapdale). Also included were data for *6-pgd*, the only allozyme system known to provide useful variation within the group, thereby seeking taxonomically meaningful correlations between morphology and genotype.

The first ordination axis separates almost completely the clouds of variation representing the three populations, demonstrating that Glenborrodale plants have larger, more reflexed labella and longer spurs but are less boldly marked than Knapdale plants. The second axis serves only to separate plants within populations according to their relative degrees of vegetative vigour, thus largely reflecting variation in the maturity of individual plants within populations rather than explicitly taxonomic trends. The morphometric plot therefore fails to separate the supposed individuals of *D. lapponica*, which have heavily spotted leaves, from those of *D. traunsteineri*, which have lightly spotted or more often unspotted leaves, in either Knapdale or Glenborrodale, which bracket the Achnaha population of 'pure' *D. lapponica.*

It was still theoretically possible that the considerable genetic variation at the *6-pgd* allozyme locus would separate the two supposed species. However, Chi-square tests show that there is no meaningful statistical correlation between any of the four genotypes recorded and the presence of heavy leaf markings; most of the variation occurs between populations rather than within them (Fig. 4). The obvious conclusion is that there is no meaningful distinction, either in morphology or allozymes, between these two supposed species, and that the British *D.* '*lapponica*' should therefore be taxonomically synoymised into *D. traunsteinerioides*. Given that *D.* '*lapponica*' does not in fact exist, it becomes a rather less compelling case for determined conservation measures (Bateman 2001).

Morphometric and population genetic data have other significant implications for the recent *New atlas of the British & Irish flora* (Preston *et al.* 2002). Although generally laudably comprehensive, this benchmark volume actually confuses on the same map (Fig. 5) UK populations of *D. ebudensis*, *D. occidentalis* and *D. purpurella* var. *cambrensis* (= *majaliformis*: Bateman & Denholm *in* Foley & Clarke 2005); taxa concentrated along the western seaboard of the British Isles that share the characteristic of being unusually rich in both floral and vegetative anthocyanins (Bateman & Denholm 1983). All three taxa are erroneously ascribed in the *Atlas* to *D. majalis s.s.*, despite the fact that each has a separate and distinct evolutionary origin, probably within the British Isles (Bateman 2001; Pillon *et al.* in press). In contrast, we finally have sufficient evidence to be confident that the distribution of so-called *D. majalis s.s.* is exclusively Continental. Lastly, the outlier of '*D. majalis*' in Yorkshire almost certainly results from introgression between *D. purpurella* and the spotted-orchids (Fig. 5).

Further innovations in the *Atlas* affected the subspecies of the diploid *D. incarnata*, which have long been taxonomically controversial; expressed opinions range from dominantly varieties (e.g. Haggar 2003–5) through dominantly subspecies (e.g. Bateman & Denholm 1985) to uniformly full species (e.g. Delforge 2001). Much of the striking morphological variation within the species actually reflects contrasts in floral and vegetative pig-

Dactylorhiza majalis Western Marsh-orchid

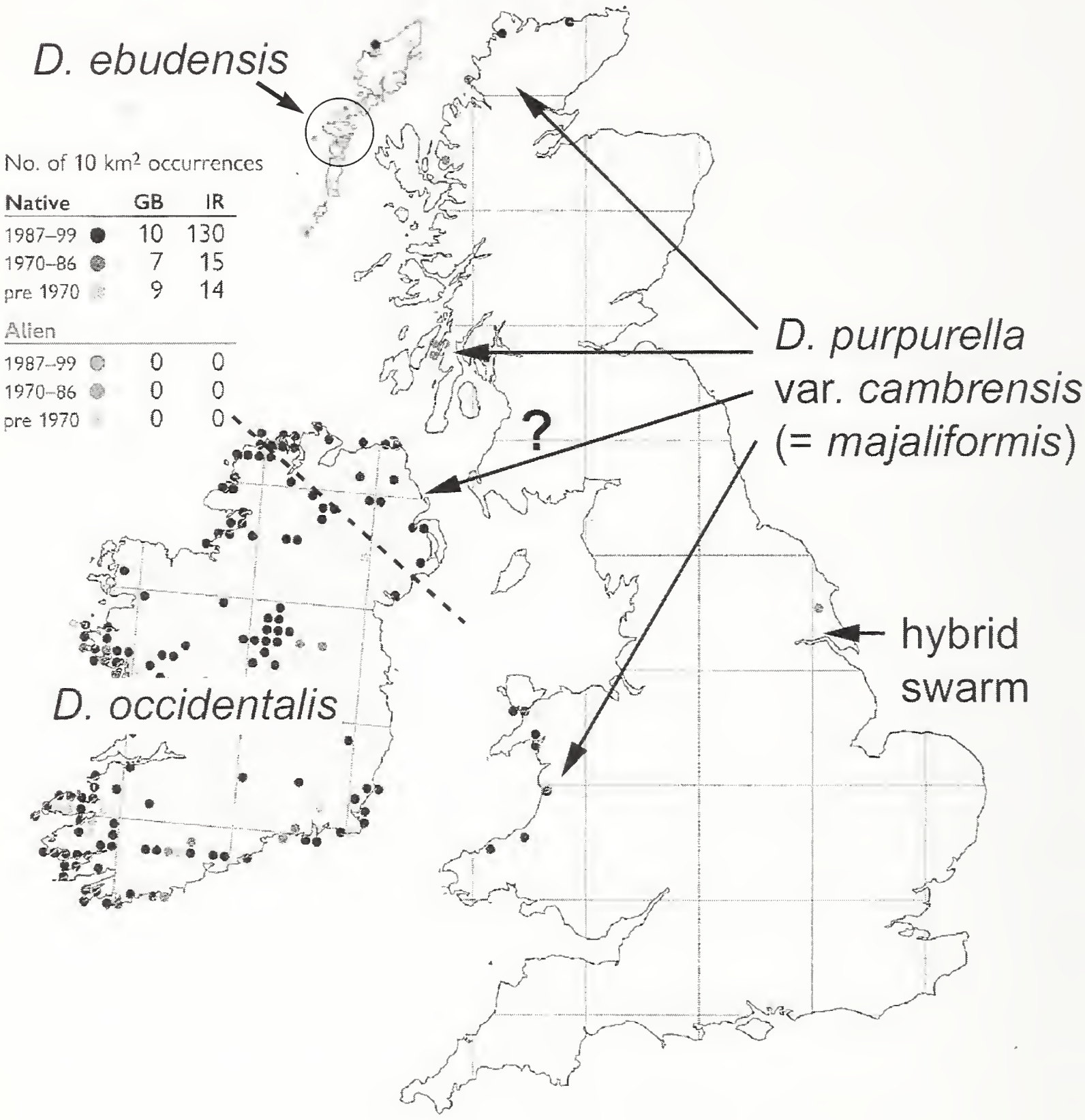

FIGURE 5. Distribution map of '*Dactylorhiza majalis*' reproduced from the *New atlas of the British & Irish flora* (Preston *et al.* 2002, p. 851). The map actually represents anthocyanin-rich individuals assignable to three allotetraploid species of independent origin within Britain, none of which corresponds with the exclusively Continental *D. majalis s.s.*

mentation, and is underpinned by remarkably little variation in allozymes (McLeod 1995; Hedrén 1996), AFLPs (Hedrén *et al.* 2001) or plastid microsatellites (Pillon *et al.* in press.). Two recently recognised localities for *D. incarnata* subsp. *cruenta* in Scotland are upheld in the *Atlas*, whereas the extraordinary decision is taken to ascribe the many classic localities for this subspecies in Eire to 'spotted-leaved variants of subsp. *pulchella*' (p. 849). In fact, *cruenta* is the one subspecies of *incarnata* that carries a distinctive allozyme marker, which is present in both the Scottish and Irish populations. No such marker picks out the equally controversial subsp. *ochroleuca*, recently recognised as Critically Endangered (Wigginton 1999; see also Foley 2000) but nonetheless unable to gain Schedule 8 status. To the evolutionary biologist (and indeed the conservation geneticist), the most notable feature of *D. incarnata s.l.* is the extraordinary lack of molecular variation to match the evident morphological variation, especially as this does not appear to have impeded the success of the species.

OTHER GENERA

British populations of *Gymnadenia* have also been subjected to recent morphological (Bateman & Denholm in prep.) and molecular (Campbell 2000; Bateman, Hollingsworth & Hollingsworth in prep.) investigations. The three named taxa that inhabit either calcareous grasslands, calcareous/neutral wetlands or acidic heathlands are reliably genetically distinct and thus could legitimately be recognised as full species, named *G. conopsea*, *G. densiflora* and *G. borealis*, respectively (cf. Colour Plate 1c–e). Although they can be difficult to distinguish from each other morphologically, and hence are often said to be cryptic, detailed morphometric studies have revealed more effective characters for separating these species; in other words, they are less cryptic than is generally supposed and, as with the dactylorchids, there should be no further barriers to accurately mapping their UK distributions during the post-*Atlas* era.

Other contrasts between molecular and morphological data are even more striking. For example, *Spiranthes romanzoffiana* is a rare species that has been considered for maximal 'Schedule 8' conservation status in the British Isles but is relatively common in North America. British and Irish populations of *S. romanzoffiana* north of the Hebridean island of Mull exhibit high levels of diversity consistent with cross-pollination, whereas populations further south show much lower genetic diversity consistent with self-pollination (Forrest *et al.* 2004). Does this contrast indicate two separate waves of immigration to the British Isles of North America seed? Or does it indicate evolutionary divergence after a single immigration event? And are there any reliable morphological differences separating the northern and southern populations? In order to answer these important questions we need additional genetic data from North America, plus morphological data from both continents, emphasising that the British and Irish flora makes evolutionary sense only when considered in a broader geographic context. In the meantime, the information already acquired on reproductive behaviour should prove of considerable use to conservation bodies.

By contrast, the two British species of butterfly-orchid, *Platanthera bifolia* and *P. chlorantha*, are readily differentiated by their appearance (Colour Plate 1a) but are extraordinarily difficult to distinguish genetically (Bateman, Rudall & James in prep.). This observation implies that they diverged only recently (if at all), despite the fact that both have become widely distributed across Eurasia. Comparison with other closely related species of *Platanthera* suggests that *P. chlorantha* probably diverged from *P. bifolia* rather than vice

versa (Bateman *et al.* 2003). Morphometric analysis demonstrates that *P. chlorantha* is about 50% larger than *P. bifolia* in most of its features, the most notable exceptions being a much wider separation of the bases of the pollinaria and a much larger spur entrance. These two characters appears to have been sufficient to dictate a switch in species of pollinating hawk-moth during the evolutionary transition from *P. bifolia* to *P. chlorantha*, constituting a potentially exceptional model system for observing the mechanism of speciation through natural selection (cf. Darwin 1877).

Equally intriguing is the differentiation between the early-flowering and late-flowering populations of *Neotinea ustulata* (cf. Colour Plate 1g, h). This is the most rapidly declining wildflower in Britain (Preston *et al.* 2002), where the larger and more widespread group of populations flowers in late May/early June, but another set of populations confined to the chalk downs of southeast England flowers in early July. Similar differentiation of populations according to flowering time is evident across the geographical range of the species, though at the eastern end of the range, in Estonia, late-flowering populations are more common than early. It has been argued that the late-flowering populations merit recognition as a new subspecies or even as a new species (in either case named *aestivalis*: Kümpel & Mrkvicka 1990), despite the fact that the supposed morphological differences are both subtle and unreliable.

Recent genetic studies of populations of *N. ustulata* across Europe, focusing on Britain and Estonia, have demonstrated that the genetic variation attributable to geographic variation is substantially greater than that attributable to any divergence between early-flowering and late-flowering populations (Tali, Fay & Bateman 2006). This implies that complete genetic isolation has not been achieved between the early-flowering and late-flowering populations. Also of interest is that fact that, in Britain, the early-flowering populations are more genetically diverse than the late-flowerers, supporting expectations that had been based on the hypothesis that the late-flowering populations diverged from the early-flowering populations and have therefore been given less time to develop genetic novelties. Other less well-known examples of supposed phenological discontinuities that occur in mainland Europe (e.g. among populations of *Anacamptis papilionacea* on Crete) would benefit from similar investigations.

MIGRATION AND EXTIRPATION

Having focused thus far on the origination of novel lineages, we should now consider two other processes that have helped determine the composition of the British orchid flora.

The first is migration, specifically by transport in air currents of the dust-like seeds of orchids. It is likely that seed of non-British orchids is constantly raining down on the British landscape, but that very few of these seeds successfully establish viable populations. This in turn is likely to be determined by whether appropriate co-evolutionary partners exist here, in the form both of viable mycorrhizal infections of their roots and (except in the cases of self-pollinated or strongly vegetatively reproducing species) of insects capable of pollinating the flowers. In this context, the certainty of global warming brings the high probability of new orchid species establishing themselves in the British Isles by northward migration from the Continent (Braithwaite *et al.* 2006). Possible examples of such migrations include the recent arrivals of *Ophrys* cf. *balearica* to Dorset, *Serapias parviflora* to Cornwall and *S. lingua* in the Channel Islands (e.g. Ettlinger 1998). Also, despite global warming, we cannot

exclude the possibility of receiving (especially in Scotland) natural migrants from boreal countries: potential immigrants include *Calypso bulbosa* and *Pseudorchis straminea.*

Expectations of natural arrivals make it imperative that deliberate introductions of such species are not attempted, as they inevitably undermine the legitimacy of natural migrants. It is also desirable that genetic fingerprinting techniques are made sufficiently precise to determine the geographic source of any surprising immigrants. Suitable case-studies for such research include the two rarest of our four unequivocally native *Ophrys* species, *O. sphegodes* and *O. fuciflora* (= *O. holoserica*); such work is already under way (Devey, Bateman & Fay in prep.). Another appropriate case-study is *Himantoglossum hircinum*, whose distribution has long been suspected of ebbing and flowing in response to regional climate change (e.g. Good 1936).

The final determining factor for us to consider is the saddest, namely extirpation – the complete loss from the British Isles of certain orchid species (this process cannot legitimately be termed extinction, as all of the species in question persist in the greener pastures of Continental Europe). It is now widely accepted that *Spiranthes aestivalis*, last seen with any frequency in the New Forest in the 1930s and finally disappearing in 1959 following a combination of draining of its marshy habitats and over-collecting by herbarium botanists, has been extirpated from the British Isles (though there are rumors of reintroductions). However, it may also be time to declare *Epipogium aphyllum* lost to the British flora (costing us a genus as well as a species); conservation databases, opinions gauged by wide consultation, and personal experience all suggest that *Epipogium* has not been seen in Britain for over a decade, thereby fully earning its colloquial name of Ghost Orchid. One possible cause of its apparent departure from the corporeal world is increasingly dry soils, reflecting both climate change and denudation of its broadleaf woodland habitats through a combination of gales and disease.

TERRESTRIAL ORCHID SPECIES, SPECIATION MECHANISMS AND CONSERVATION

Taken together, these combined genetic and morphological studies demonstrate that we in Britain play host to a range of different *kinds* of orchid species (Bateman 2001; Hollingsworth 2003). Some supposed species do not withstand close scientific scrutiny, possessing neither morphological nor molecular cohesion. Examples in Britain include some of our greatest supposed 'rarities': *Epipactis* '*youngiana*', *Dactylorhiza* '*lapponica*' and, arguably, the late-flowering *Neotinea ustulata* 'subsp.' *aestivalis.* These unjustified 'species', best described as 'Emperor's New Clothes' species, have in the past inflicted a substantial (and avoidable) drain on conservation resources. Other species, such as the three contrasting habitat specialists that have evolved within *Gymnadenia*, show greater molecular differences than morphological differences; consequently, they have not yet become widely recognised as full species, despite meriting such distinction. Such plants are 'Cinderella' species.

A subcategory of Cinderella species are 'Robinson Crusoe' species: geographically isolated populations that have acquired their own characteristic genetic motif, together with a morphological spectrum that is at least subtly distinct from their closest relatives. Examples mentioned above include *Epipactis sancta* from Lindisfarne and *Dactylorhiza ebudensis* from North Uist. We can also expect to become unwitting hosts of increasing numbers of 'Bleriot' species such as *Serapias parviflora*, which may have flown across the English Channel unaided but alternatively could have been given unwarranted assistance by man.

Nonetheless, it may appear that the majority of British orchid species have long been widely recognised and are uncontroversial. But then, many of our orchids have not yet been subjected to intense combined morphological and molecular scrutiny (cf. Bateman 2001; Shipunov *et al.* 2004; Shipunov & Bateman 2005). I therefore confidently predict that further surprises await us. One conclusion rapidly and clearly emerging from recent research is that a wide range of evolutionary processes cause speciation in temperate orchids. Thus far, each of our detailed case-studies has implicated a different causal mechanism; for example, allopolyploidy in *Dactylorhiza ebudensis*, mutationally driven autogamy in *Epipactis dunensis*, mutationally driven pollinator divergence in *Platanthera chlorantha*, and increase in habitat tolerance followed by specialisation in the *Gymnadenia conopsea* group (Colour Plate 1). Moreover, not all speciation events confer immediate adaptive advantage; it would seem that evolution is more complex, and thus more interesting, than even Darwin could have predicted.

Furthermore, we still have some very challenging questions to collectively answer. Which combination of the many analytical techniques now available is most effective for circumscribing species? Must taxa be both morphologically and molecularly distinct in order to be deemed legitimate species? If not, which of the two kinds of data should be prioritised? If small geographically peripheral populations, or occasional radically altered mutant forms within populations (including self-pollinating lines), fulfil both these criteria, are they sufficiently abundant and/or persistent to warrant recognition as species? Should multiple origins of self-pollinating species from a single cross-pollinating parental species, or of allopolyploid species from the same pair of parental species, each be differentiated as separate species despite their inevitable morphological similarity? How can conservationists make best use of the burgeoning database of evolutionary knowledge? And, lastly, will they be prepared to abandon long-cherished supposed species that in fact have no biological reality, or to re-evaluate the scientific wisdom of re-introduction programmes?

The conservation implications of the recent research summarised in this paper will be discussed in a more detailed companion paper (Bateman in prep.). One key point to note here is the consequences of the taxonomic uncertainties that surround putative endemics. The only supposed endemic orchid protected under Schedule 8 is *Epipactis* '*youngiana*', which in fact lacks biological reality. In contrast, a fairly widespread *bona fide* endemic, *E. dunensis*, was dropped from the most recent *Red Data Book* (Wigginton 1999) (but see Note added in proof). Another more localised endemic, the recently described *E. sancta*, has yet to be awarded any form of conservation status, as has the equally localised endemic *Dactylorhiza ebudensis*. British populations of *Dactylorhiza* '*lapponica*' are judged 'Near-Threatened' and appear in the *Red Data Book* (Wigginton 1999), whereas *D.* '*traunsteineri*' is judged 'Scarce' and must be content with inclusion in *Scarce plants in Britain* (Stewart *et al.* 1994). In fact, these two taxa are conspecific in Britain (Bateman 2001), but together they have a separate evolutionary origin from the true *D. traunsteineri* in the Alps, which in turn has a separate evolutionary origin from the true *D. lapponica* in Scandinavia (e.g. Pillon *et al.* in press). Both British taxa can therefore be ascribed to *D. traunsteinerioides*, which does appear to be endemic to the British Isles, but is not presently recognised in any prominent conservation summary of the British and Irish flora (cf. Stewart *et al.* 1994; Wigginton 1999; Preston *et al.* 2002).

These and other similar case-histories demonstrate conclusively that well-integrated systematic studies are an essential pre-requisite for species conservation, rather than being relegated to a more post-hoc procedure expected to verify 'conventional wisdom.'

CONCLUSION

Despite the many codicils outlined above, some readers may still desire an explicit answer to the ostensibly simple question posed in the title of this article. I therefore feel honour-bound to attempt a response. If *Spiranthes aestivalis* and *Epipogium aphyllum* are judged to be extirpated from the British Isles, if *Anacamptis* (formerly *Orchis*) *laxiflora* and *Serapias lingua* on the Channel Islands are viewed in a biogeographic context as being French rather than English, and if the rare Robinson Crusoe species *Dactylorhiza ebudensis* and *Epipactis sancta* are regarded as acceptable (natural but 'neophyte') species, the British orchid flora currently consists of 52 species in 20 genera. Appendix 1 reclassifies these taxa in anticipation of the third edition of Stace's *New Flora of the British Isles* (cf. Stace 1997). It has already been used as the framework for two of the three recent orchid Floras of the British Isles (Foley & Clarke 2005; Harrup & Harrup 2005; *contra* Lang 2004).

But this figure is not fixed, nor should it ever be considered fixed; it is essential that systematics should remain a dynamic science capable of delivering further surprises, even to students of the world's best-known flora. The rigour of recent studies of British and Irish orchids is now being applied to other plant families, and similarly startling results can be predicted. Our increasing understanding of the complexities and vagaries of evolutionary biology will inevitably prove difficult to shoe-horn into the spurious precision currently advocated for 'biodiversity accountancy.'

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REFERENCES

BATEMAN, R.M. (1999). Integrating molecular and morphological evidence for evolutionary radiations. In: P.M. HOLLINGSWORTH, R.M. BATEMAN & R.J. GORNALL (eds), *Molecular systematics and plant evolution*, pp. 432–471. Taylor & Francis, London.

BATEMAN, R.M. (2001). Evolution and classification of European orchids: insights from molecular and morphological characters. *Journal Europäischer Orchideen* **33**: 33–119.

BATEMAN, R.M. (2004). Burnt tips and bumbling bees: how many orchid species currently occur in the British Isles? *Journal of the Hardy Orchid Society* **1**: 10–18.

BATEMAN, R.M. (in press). She's no Lady! A hybrid orchid new to the British Isles. *Orchid Review* **115**.

BATEMAN, R.M. & DENHOLM, I. (1983). A reappraisal of the British and Irish dactylorchids, 1. The tetraploid marsh-orchids. *Watsonia* **14**: 347–376.

BATEMAN, R.M. & DENHOLM, I. (1985). A reappraisal of the British and Irish dactylorchids, 2. The diploid marsh-orchids. *Watsonia* **15**: 321–355.

BATEMAN, R.M. & DENHOLM, I. (2003). The Heath Spotted-orchid (*Dactylorhiza maculata* (L.) Sóo) in the British Isles: a cautionary case-study in delimitating infraspecific taxa and inferring their evolutionary relationships. *Journal Europäischer Orchideen* **35**: 3–36.

BATEMAN, R.M. & HOLLINGSWORTH, P.M. (2004). Morphological and molecular investigation of the parentage and maternity of *Anacamptis* ×*albuferensis* (*A. fragrans* × *A. robusta*), a new hybrid orchid from Mallorca, Spain. *Taxon* **53**: 43–54.

BATEMAN, R.M., HOLLINGSWORTH, P.M., PRESTON, J., LUO YI-BO, PRIDGEON, A.M. & CHASE, M.W. (2003). Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). *Botanical Journal of the Linnean Society* **142**: 1–40.

BATEMAN, R.M., HOLLINGSWORTH, P.M., SQUIRRELL, J. & HOLLINGSWORTH, M. (2005). Phylogenetics: Neottieae. In: A.M. PRIDGEON, P.J. CRIBB, M.W. CHASE & F.N. RASMUSSEN (eds), *Genera Orchidacearum, 4. Epidendroideae 1*, pp. 487–495. Oxford University Press.

BATEMAN, R.M., RUDALL, P.J. & JAMES, K.E. (2006). Phylogenetic context, generic affinities and evolutionary origin of the enigmatic Balkan orchid *Gymnadenia frivaldii* Hampe ex Griseb. *Taxon* **55**: 107–118.

BIDARTONDO, M.I., BURGHARDT, B., GEBAUER, G., BRUNS, T.D. & READ, D.J. (2004). Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. *Proceedings of the Royal Society of London B* **271**: 1799–1806.

BRAITHWAITE, M.E., ELLIS, R.W. & PRESTON, C.D. (2006). *Change in the British flora 1987–2004*. BSBI, London.

CAMERON, K.M., CHASE, M.W., WHITTEN, W.M., KORES, P.J., JARRELL, D.C., ALBERT, V.A., YUKAWA, T., HILLS, H.G. & GOLDMAN, D.H. (1999). A phylogenetic analysis of the Orchidaceae: evidence from *rbc*L nucleotide sequences. *American Journal of Botany* **86**: 208–244.

CAMPBELL, V. (2000). *Aspects of molecular ecology and population biology of* Gymnadenia conopsea *(L.) R.Br. in Britain.* PhD thesis, University of Sussex.

COX, A.V., PRIDGEON A.M., ALBERT, V.A. & CHASE, M.W. (1997). Phylogenetics of the slipper orchids (Cypripedioideae: Orchidaceae): nuclear rDNA sequences. *Plant Systematics and Evolution* **208**: 197–223.

DARWIN, C. (1877). *The various contrivances by which orchids are fertilised by insects* (2nd edn). Murray, London.

DELFORGE, P. (2001). *Guide des Orchidées d'Europe* (edn 2). Delachaux et Niestlé, Lausanne, Switzerland.

DELFORGE, P. & GÉVAUDAN, A. (2002). Contribution taxonomique et nomenclaturale au groupe d' *Epipactis leptochila*. *Les Naturalistes belges, hors-série – spécial Orchidées* **83** (Orchid 15): 19–35.

DEVOS, N., TYTECA, D., RASPÉ, O., WESSELINGH, R.A. & JACQUEMART, A.-L. (2004). Pattern of chloroplast diversity among western European *Dactylorhiza* (Orchidaceae). *Plant Systematics and Evolution*.

DRESSLER, R.L. (1993). *Phylogeny and classification of the orchid family*. Portland, Oregon: Timber Press.

ETTLINGER, D.M.T. (1998). *Illustrations of British and Irish orchids*. Royden Cottage, Dorking, Surrey, U.K. (self-published).

FOLEY, M.J.Y. (2000). *Dactylorhiza incarnata* (L.) Soó subsp. *ochroleuca* (Wüstnei ex Boll) P.F. Hunt & Summerh. (Orchidaceae): a comparison of British and European plants. *Watsonia* **23**: 299–303.

FOLEY, M.S.Y & CLARKE, S. *Orchids of the British Isles*. Griffin Press, Cheltenham, Gloucs.

FORREST, A.D., HOLLINGSWORTH, M.L., HOLLINGSWORTH, P.M., SYDES, C. & BATEMAN, R.M. (2004). Population genetic structure in European populations of *Spiranthes romanzoffiana* set in the context of other genetic studies on orchids. *Heredity* **92**: 218–227.

FREUDENSTEIN, J.V. & RASMUSSEN, F.N. (1999). What does morphology tell us about orchid relationships? A cladistic analysis. *American Journal of Botany* **86**: 225–248.

GOOD, R. (1936). On the distribution of the Lizard Orchid (*Himantoglossum hircinum* Koch). *New Phytologist* **35**: 142–170.

HAGGAR, J. (2003–5). The Early Marsh Orchid in northern Europe. *Journal of the Hardy Orchid Society* **27**: 4–9, **29**: 17–22, **31**: 18–23, **32**: 45–51, **36**: 51–58, **38**: 116–123.

HARRAP, A. & HARRAP, S. (2005). *Orchids of Britain and Ireland*. A & C Black, London.

HARRIS, S.A. & ABBOTT, R.J. (1997). Isozyme analysis of the reported origin of a new hybrid orchid species, *Epipactis youngiana* (Young's Helleborine) in the British Isles. *Heredity* **79**: 402–407.

HEDRÉN, M. (1996). Genetic differentiation, polyploidization and hybridization in northern European *Dactylorhiza* (Orchidaceae): evidence from allozyme markers. *Plant Systematics and Evolution* **201**: 31–55.

HEDRÉN, M. (2001). Systematics of the *Dactylorhiza euxina/incarnata/maculata* polyploid complex (Orchidaceae) in Turkey: evidence from allozyme data. *Plant Systematics and Evolution* **229**: 23–44.

HEDRÉN, M. (2003). Plastid variation in the *Dactylorhiza incarnata/maculata* polyploid complex and the origin of allotetraploid *D. sphagnicola* (Orchidaceae). *Molecular Ecology* **12**: 2669–2680.

HEDRÉN, M., FAY, M.F. & CHASE, M.W. (2001). Amplified fragment length polymorphisms (AFLP) reveal details of polyploid evolution in *Dactylorhiza* (Orchidaceae). *American Journal of Botany* **88**: 1868–1880.

HOLLINGSWORTH, P.M. (2003). Taxonomic complexity, population genetics, and plant conservation in Scotland. *Botanical Journal of Scotland* **55**: 55–63.

HOLLINGSWORTH, P.M., SQUIRRELL, J, HOLLINGSWORTH, M.L., RICHARDS, A.J. & BATEMAN, R.M. (2006). Taxonomic complexity, conservation and recurrent origins of self-pollination in *Epipactis* (Orchidaceae). In J. BAILEY & G. ELLIS (eds) *Current taxonomic research on the British & European flora*, pp. 27–44. BSBI, London.

KÜMPEL, H. & MRKVICKA, A.C. (1990). Untersuchungen zur Abtrennung der *Orchis ustulata* subsp. *aestivalis* (Kümpel) Kümpel & Mrkvicka. *Jahresbericht naturw-isschaften Vereins Wuppertal* **22**: 306–324.

LANDWEHR, J. (1977). *Wilde Orchideeën van Europa*. Natuurmonumentum, Amsterdam.

LANG, D.C. (2004). *Britain's Orchids*. WILD Guides, Old Basing, Hants.

MCLEOD, L. (1995). *Combined morphometric and isozyme analysis of* Dactylorhiza *in Scotland*. MSc thesis, Royal Botanic Garden Edinburgh and Edinburgh University.

PEDERSEN, H. A. (1998). Species concept and guidelines for infraspecific taxonomic ranking in *Dactylorhiza* (Orchidaceae). *Nordic Journal of Botany* **18**: 289–310.

PILLON, Y., FAY, M.F., HEDRÉN, M., BATEMAN, R.M., DEVEY, D.S., SHIPUNOV, A.B., VAN DER BANK, M. & CHASE, M.W. (in press). Insights into the evolution and biogeography of western European species complexes in *Dactylorhiza* (Orchidaceae). *Taxon*.

PRESTON, C.D., PEARMAN, D.A. & DINES, T.D. (2002). *New atlas of the British & Irish flora*. Oxford University Press, Oxford.

QAMARUZ-ZAMAN, F. (2000). *Conservation genetics of rare and endangered British orchids*. Unpublished doctoral thesis, University of Cambridge/Royal Botanic Gardens, Kew.

SALAZAR, G.A., CHASE, M.W., SOTO ARENAS, M.A. & INGROUILLE, M. (2003). Phylogenetics of Cranichideae with emphasis on Spiranthinae (Orchidaceae: Orchidoideae): evidence from plastid and nuclear DNA sequences. *American Journal of Botany* **90**: 777–795.

SHIPUNOV, A.B. & BATEMAN, R.M. (2005). Geometric morphometrics as a tool for understanding *Dactylorhiza* (Orchidaceae) diversity in European Russia. *Biological Journal of the Linnean Society* **85**: 1–12.

SHIPUNOV, A.B., FAY, M.F., PILLON, Y., BATEMAN, R.M. & CHASE, M.W. (2004). *Dactylorhiza* (Orchidaceae) in European Russia: combined molecular and morphological analysis. *American Journal of Botany* **91**: 1419–1426.

SOLIVA, M., KOCYAN, A. & WIDMER, A. (2001). Molecular phylogenetics of the sexually deceptive orchid genus *Ophrys* (Orchidaceae) based on nuclear and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* **20**: 78–88.

SQUIRRELL, J., HOLLINGSWORTH, P.M., BATEMAN, R.M., DICKSON, J.H. LIGHT, M.H.S., MCCONAILL, M. & TEBBITT, M.C. (2001). Partitioning and diversity of nuclear and organelle markers in native and introduced populations of *Epipactis helleborine* (Orchidaceae). *American Journal of Botany* **88**: 1409–1418.

SQUIRRELL, J., HOLLINGSWORTH, P.M., BATEMAN, R.M., TEBBITT, M.C. & HOLLINGSWORTH, M.L. (2002). Taxonomic complexity and breeding system transitions: conservation genetics of the *Epipactis leptochila* complex. *Molecular Ecology* **11**: 1957–1964.

STACE, C.A. (1975). *Hybridization and the British flora*. Academic Press, London.

STACE, C.A. (1989). *Plant taxonomy and biosystematics* (2nd edn). Arnold, London.

STACE, C.A. (1997). *New flora of the British Isles* (2nd edn). Cambridge Univ. Press, Cambridge.

STEWART, A., PEARMAN, D.A. & PRESTON, C.D. (1994). *Scarce plants in Britain*. JNCC, Peterborough.

TALI, K., FAY, M.F. & BATEMAN, R.M. (2006). Little genetic differentiation across Europe between early-flowering and late-flowering populations of the rapidly declining orchid *Neotinea ustulata*. *Biological Journal of the Linnean Society* **87**: 13–25.

WIGGINTON, M.J. (1999). *British red data books: 1 Vascular plants*. JNCC, Peterborough.

APPENDIX 1. RECLASSIFICATION OF THE UK ORCHID FLORA IN THE LIGHT OF RECENT PHYLOGENETIC, POPULATION GENETIC AND MORPHOMETRIC STUDIES.

Cypripedioideae

1. *Cypripedium* L.

1.1 ***C. calceolus*** L. [Lady's-slipper]

Epidendroideae

Neottieae

2. *Cephalanthera* LCM Rich.

2.1 ***C. rubra*** (L.) LCM Rich. [Red Helleborine]

2.2 ***C. longifolia*** (L.) Fritsch [Narrow-leaved Helleborine]

2.3 ***C. damasonium*** (Miller) Druce [White Helleborine]

3. *Neottia* Guett. (incl. *Listera* R Br. ex WT Aiton)

3.1 ***N. (Listera) cordata*** (L.) LCM Rich. [Lesser Twayblade]

3.2 ***N. (Listera) ovata*** (L.) Bluff. & Fingerh. [Common Twayblade]

3.3 ***N. nidus-avis*** (L.) LCM Rich. [Bird's-nest Orchid]

4. *Epipactis* Zinn.

palustris group

4.1 ***E. palustris*** (L.) Crantz [Marsh Helleborine]

helleborine group

4.2 ***E. atrorubĕns*** (Hoffm. ex Bernh.) Besser [Dark-red Helleborine]

4.3 ***E. helleborine*** (L.) Crantz [Broad-leaved Helleborine] {inc. *E. youngiana* Richards & Porter}

4.4 ***E. purpurata*** Smith [Violet Helleborine]

4.5 ***E. leptochila*** (Godfery) Godfery [Narrow-lipped Helleborine]

4.6 ***E. dunensis*** (T & TA Steph.) Godfery [Dune Helleborine]

4.7 ***E. phyllanthes*** GE Smith [Green-flowered Helleborine]

4.8 ****E. sancta*** (P Delforge) P Delforge & A Gévaudan [Lindisfarne Helleborine] {**very localised**}

?Gastrodieae

Epipogium Gmelin ex Borkh.

E. aphyllum Swarz [Ghost Orchid] {**apparently extirpated in the British Isles**}

Malaxideae

5. *Liparis* LCM Rich.

5.1 ***L. loeselii*** (L.) LCM Rich. [Fen Orchid] {inc. *ovata*}

6. *Hammarbya* Kuntze

6.1 ***H. paludosa*** (L.) Kuntze [Bog Orchid]

Calypsoeae

7. *Corallorhiza* Ruppius ex Gagnebin

7.1 ***C. trifida*** Chatel. [Coralroot Orchid]

Orchidoideae

Cranichideae

Goodyerinae

8. *Goodyera* R Br.

8.1 ***G. repens*** (L.) R Br. [Creeping Lady's-tresses]

Spiranthinae
9. *Spiranthes* LC Rich.
9.1 ***S. romanzoffiana*** Cham. [Irish Lady's-tresses]
9.2 ***S. spiralis*** (L.) Chevall. [Autumn Lady's-tresses]
S. aestivalis (Poiret) LCM Rich. [Summer Lady's-tresses]
{**extirpated in the British Isles**}

Orchideae
Habenariinae
10. *Herminium* L.
10.1 ***H. monorchis*** (L.) R Br. [Musk Orchid]

Orchidinae
Orchis clade
11. *Orchis* L. *s.s.* (inc. *Aceras* R Br.)
militaris group
11.1 ***O. (Aceras) anthropophora*** (L.) All. [Man Orchid]
11.2 ***O. simia*** Lam. [Monkey Orchid]
11.3 ***O. militaris*** L. [Military Orchid]
11.4 ***O. purpurea*** Hudson [Lady Orchid]
mascula group
11.5 ***O. mascula*** (L.) L. [Early-purple Orchid]

Platanthera clade
12. *Pseudorchis* Seguier
12.1 ***Ps. albida*** (L.) A & D Loeve [Small-white Orchid]
13. *Platanthera* LCM Rich.
13.1 ***P. bifolia*** (L.) LCM Rich. [Lesser Butterfly-orchid]
13.2 ***P. chlorantha*** (Custer) Reichb. p. [Greater Butterfly-orchid]

Gymnadenia clade
14. *Gymnadenia* R Br. s.l. {inc. *Nigritella* LCM Rich.}
14.1 ***G. conopsea*** (L.) R Br. [Chalk Fragrant-orchid]
14.2 ***G. borealis*** (Druce) RM Bateman, Pridgeon & MW Chase [Heath Fragrant-orchid]
14.3 ***G. densiflora*** (Wahlenb.) Dietrich [Marsh Fragrant-orchid]

Dactylorhiza clade
15. *Dactylorhiza* Necker ex Nevski *s.l.* {inc. *Coeloglossum* Hartm.}
incarnata group
15.1 ***D. incarnata*** (L.) Soó {inc. *cruenta*} [Early Marsh-orchid]
viridis group
15.2 ***D. (Coeloglossum) viridis*** (L.) RM Bateman, Pridgeon & MW Chase [Frog Orchid]
fuchsii group
15.3 ***D. fuchsii*** (Druce) Soó [Common Spotted-orchid]
maculata group
15.4 ***D. maculata*** (L.) Soó [Heath Spotted-orchid]
majalis s.l. group
15.5 ***D. praetermissa*** (Druce) Soó [Southern Marsh-orchid] {inc. *junialis*}
15.6 ***D. traunsteinerioides*** (Pugsley) Landwehr ex RM Bateman & Denholm [Pugsley's Marsh-orchid] {inc. *lapponica*}

15.7 ***D. ebudensis*** (Wiefelspuetz ex RM Bateman & Denholm) P Delforge [Hebridean Marsh-orchid] {**very localised**}
15.8 ***D. purpurella*** (T & TA Steph.) Soó [Northern Marsh-orchid] {incl. *cambrensis* = *majaliformis*}
15.9 ***D. occidentalis*** (Pugsley) P Delforge [Irish Marsh-orchid] {incl. *kerryensis*}

Neotinea clade
16. *Neotinea* Reichb. f. *s.l.*
maculata group
16.1 ***N. maculata*** (Desf.) Stearn [Dense-flowered Orchid] {**Eire only**}
ustulata group
16.2 ***N. (Orchis) ustulata*** (L.) RM Bateman, Pridgeon & MW Chase [Burnt Orchid] {inc. *aestivalis*}

Himantoglossum clade
17. *Himantoglossum* Koch *s.l.*
17.1 ***H. hircinum*** (L.) Sprengel [Lizard Orchid]

Anacamptis clade
18. *Anacamptis* LCM Rich. *s.l.*
laxiflora group
A. (Orchis) laxiflora (Lam.) RM Bateman, Pridgeon & MW Chase [Loose-flowered Orchid] {**Channel Isles only**}
pyramidalis group
18.1 ***A. pyramidalis*** (L.) LCM Rich. [Pyramidal Orchid]
morio group
18.2 ***A. (Orchis) morio*** (L.) RM Bateman, Pridgeon & MW Chase [Green-winged Orchid]

Serapias clade
19. *Serapias* L.
19.1 ***S. parviflora*** Parlatore [Lesser Tongue-orchid] {**questionably native**}
S. lingua L. [Greater Tongue-orchid] {**Channel Isles only**}

Ophrys clade
20. *Ophrys* L.
insectifera group
20.1 ***O. insectifera*** L. [Fly Orchid]
apifera group
20.2 ***O. apifera*** Hudson [Bee Orchid]
fuciflora–sphegodes group
20.3 ***O. fuciflora*** (Crantz) Moench *s.s.* [Late Spider-orchid]
20.4 ***O. sphegodes*** Miller *s.s.* [Early Spider-orchid]

Total = 20 genera (including *Serapias* but excluding *Epipogium*); 50 unequivocal species + 2 prospecies + 2 extirpated species + 2 species found only in the Channel Isles

Note that several of the species have recently been, or are, the subject of nomenclatural debates focused on the rules of priority (e.g. *Epipactis purpurata*, *Pseudorchis albida*, *Platanthera chlorantha*, *Gymnadenia densiflora*, *Dactylorhiza viridis* (also the Continental *D. majalis*), *Neotinea maculata*, *Ophrys fuciflora*).

NOTE ADDED IN PROOF

Although the paragraph on the conservation status of UK orchids presented on p. 102 accurately represents the situation that pertained at the time of the Stace Conference in 2003, the situation was substantially altered by the subsequent publication of the fourth edition of the Red Data List (Cheffings *et al.* 2005). The classification adopted in the latest Red List was informed by, and indeed precisely reflects, that presented here (with appropriate acknowledgement to this manuscript on p. 17).

Spiranthes aestivalis is once again considered Extinct (strictly, extirpated) but so, more controversially, is *Epipogium aphyllum*. The Cornish *Serapias parviflora* population is placed in conservational limbo under a watching brief, to await evidence of successful expansion.

Epipactis and *Dactylorhiza* constitute two of the 11 'critical groups' recognised in the Red Data List. Justly, *E. sancta* is designated Endangered while *E.* '*youngiana*' is omitted. However, *E. dunensis* and *E. leptochila* are surprisingly placed in the 'pending' category, termed Data Deficient. Even more surprising assignees to this category are *D. incarnata* subsp. *cruenta* and subsp. *ochroleuca*, since each is widely recognised by the orchidological community as being represented in mainland Britain by only two or three small populations. *Dactylorhiza ebudensis* is designated Vulnerable due to its highly restricted distribution as an endemic of North Uist. Although *D.* '*lapponica*' is correctly sunk into *D. traunsteinerioides*, its lowly designation as Least Concern seems surprising in view of the vulnerability of its preferred habitat, calcareous flushes.

Both *Epipactis* and *Dactylorhiza* are viewed by Cheffings *et al.* (2005) as actively evolving 'taxonomically complex groups' and so are deemed suitable cases for 'process-based conservation' *sensu* Ennos *et al.* (2005). Although this approach appears attractive at a conceptual level, the author remains sceptical about its practicality. For Orchidaceae at least, such complex populations tend to occupy heavily anthropogenic habitats that are subject to rapid change. This instability in turn renders the orchid populations relatively transient unless they receive substantial, open-ended intervention from conservationists – an approach that is liable to fail the criterion of cost-effectiveness.

CHEFFINGS, C.M., FARRELL, L., plus eight coauthors (2005). The vascular plant Red Data List for Great Britain. *Species Status* **7**: 1–116. JNCC, Peterborough.

ENNOS, R.A., FORREST, G.C. & HOLLINGSWORTH, P.M. (2005). Conserving taxonomic complexity. *Trends Ecol. Evol.* **20**: 164–168.

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Factors affecting hybridisation from GM oilseed rape in the United Kingdom

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ABSTRACT

On a global scale, the cultivation of genetically modified (GM) crops has risen dramatically over the past five years and now stands at almost 60Mha. Whilst there has been no commercial release of GM crops in the UK to date, the government is currently reviewing the situation and releases are possible within a relatively short time frame. Concerns over the possible consequences of widespread use of GM crops centre on the possible risks to human health and to the environment. The latter range from indirect effects such as those caused by changed farm practice or agricultural market forces through to direct impacts of a transgene either on the crop itself outside cultivation or on weedy or wild relatives following gene flow. In this talk, I shall use the possibility of unwanted environmental change arising from gene flow between GM oilseed rape and natural populations of *Brassica rapa* to illustrate the complexity of assessing risks on a National scale.

THE RANGE OF RISKS POSED BY GENETICALLY MODIFIED CROPS

The commercialisation of Genetically Modified (GM) crops is a topic that evokes strong and often polarised views amongst scientists and public alike. Controversy stems from the balance between the benefits that may accrue from exploitation of the technology and the possible risks that could arise from the widespread cultivation of such material. In Europe, as elsewhere, the decision-making process for the commercial release of GM cultivars is based on a case-by-case evaluation of the risks posed by each cultivar within the national setting. We can broadly divide risks relating to GM crops into those associated with possible effects on human or animal health, and those impacting on the wider environment. Interest in health risks centre largely on possible exposure of animals or humans to the protein products of the transgene (e.g. Lack 2002) and are not be examined here. The risks posed by GM crops into the wider environment are more complex to identify and much more difficult to quantify. There are three areas of concern that warrant attention: implications of changed farm practice; economic consequences of crop-to-crop gene flow; and the possible ecological consequences following transgene movement into wild relatives of GM crop plants.

The scope for changed farm practice is heavily dependent upon the sort of transgene(s) that are present in the crop. To date, most attention has focused on the prospect for altered herbicide application patterns in GM herbicide Tolerant (HT) crops or on changed pesticide use in insect-resistant GM cultivars. Such changes undoubtedly have the capacity to significantly disturb the species composition of on-farm communities, as was illustrated in the seminal Farm Scale Evaluation (FSE) study of 2000-2004. In this study, direct empirical observations of species abundance and composition was compared in GM and non-GM equivalent crops occupying adjacent halves of the same field, with the number of sites

replicated in 60-75 locations across the UK (Squire *et al.* 2003). The aim was to test whether GM herbicide tolerant sugar beet, maize and oilseed rape has a greater effect on on-farm biodiversity than a conventional cropping system. This design allowed direct comparisons of the abundance and diversity of the in-field flora and fauna. The data generated had great statistical power and was of direct relevance for the decision-making process of the UK regulators. Overall, the study found that within-field biodiversity declined in GM oilseed rape and GM HT beet relative to their non-GM counterparts but had increased in GM HT maize. This finding undoubtedly played a key role subsequent UK government policy decisions.

Similar attention has been given to the possibility of transgene movement from a GM cultivar to a non-GM cultivar of the same crop via conspecific hybridisation. In this instance, concern surrounds the effect that transgene movement has on the market value of the non-GM crop (particularly in the case of organic growers) or on the possibility of crop volunteer weeds assimilating several transgenes that confer tolerance to alternative herbicides. Whilst there have been numerous attempts to predict gene flow rates between fields using modelling approaches (Giddings 2000; Loos *et al.* 2003), it was Reiger and colleagues (2002) that provided the first large-scale empirical estimate of inter-field hybridisation rates between GM and non-GM fields of oilseed rape in Australia during the first year of GM cultivation. Friesen *et al.* (2003) provided complementary information relating to the likelihood of transgenes appearing in non-GM cultivars through the accidental mixing of seed stocks. Such data clearly have economic and political importance, and provide a valuable reference point that aids regulators in the decision-making process. Their ecological significance on the other hand is somewhat limited.

It is the possible implications of gene flow between GM crops and wild relatives that present the greatest challenge to those that are active in risk assessment research. This is chiefly because of the large number of different ways in which hybrid formation could ultimately lead to unwanted ecological perturbation. This article surveys the problems associated with assembling a comprehensive assessment of risks relating to gene flow from GM crops to wild relatives and highlights strategies that could be adopted to ensure that the data assembled has generic value for the regulatory procedure. The scenario of predicting the consequences of gene flow from oilseed rape to wild relatives in the UK will be used as an illustrative example.

THE OILSEED RAPE CROP

Oilseed rape (*B. napus* ssp. *oleifera*) is a member of *Brassicaceae.* It is a dibasic allotetraploid crop (2n=4x=38, AACC) and is believed to have originated in cultivation from the spontaneous hybridisation between *Brassica rapa* (2n=2x=20) and *Brassica oleracea* (2n=2x=18) somewhere in Southern Europe (Song & Osborn 1992). The crop is cultivated throughout Europe, North America, China and the Indian subcontinent for the oil in its seeds. Within the United Kingdom, it is used principally as a break crop for cereals and has the largest acreage of non-cereal crops. Oilseed rape is particularly amenable to genetic modification and was one of the first crops to be genetically modified after the initial studies on model plants (Ooms *et al.* 1985). It is one of the three crops currently being considered for approval for the release of GM cultivars in the UK; the others are sugar beet and maize.

THE NEED TO MEASURE HYBRID FREQUENCY

Risk is usually defined by the formula: Risk = *f* (hazard, exposure). When attempting to evaluate the risks of an undesirable change to the environment (hazard), it is important to define the hazard in terms that can be measured. The phrase 'unwanted change' is completely inadequate in this sense as it is open to several interpretations. A more fitting example definition of a specific hazard would be 'transgene movement from crop to wild relative ultimately leading to decline or extinction of a named organism'. In this sense, we can measure decline and extinction of a specified organism. Difficulty resides in the many possible organisms that could be named in this example, and also many other specific changes to the environment that could equally be classified as unwanted. It is this abundance of possible hazards that complicates assessment of risks relating to gene flow. This can be addressed to some extent by considering the exposure element of the formula. Exposure is a measure of the likelihood that a particular hazard will occur. In the case of hazards relating to gene flow, all hazards share many components of exposure in common. In essentially all cases, for instance, it is necessary for hybrids to form, for the transgene to stabilize by introgression and for the transgene to spread before hazards can be realized. Hybrid formation is the first of these elements and so is the most important to measure. Moreover, if no hybrids are expected in a geographically defined region, then none of the hazards relating to gene flow can occur. The same is true if the frequency of hybrids can be sufficiently depressed by risk-management procedures so that no hybrids are expected within a given time frame. Indeed, several workers have proposed strategies to reduce or eliminate hybrid formation between GM crops and wild relatives. These include the enforcement of isolation distances (Waines & Hedge, 2003), the use of male sterile GM lines (Rosellini *et al.* 2001), selection of 'safe' integration sites for a transgene (Metz *et al.*1997), transformation of the chloroplast (Daniell *et al.* 1999) and the use of inducible promotor systems, popularly termed 'terminator technology' (Oliver *et al.* 1999). These approaches are likely to vary dramatically in their ability to depress hybrid abundance and so may or may not be effective in managing the risk of gene flow. A broad estimate of hybrid abundance in a legislative region (in this case the national scale) over a given time period is therefore needed to define how effective such measures must be to prevent hybrid formation or to repress hybrid abundance sufficiently to render transgene spread improbable.

PREDICTING HYBRID ABUNDANCE AND DISTRIBUTION

Hybrid abundance is influenced by many factors including the strength of interspecific breeding barriers, proximity and context of contact between the crop and populations of the wild relative, and on the pollen dispersal characteristics of the crop. These factors are predominantly independent of the transgene but will vary according to crop-recipient combination, geographic scale, and the predominant farming practice employed in the region. For any defined geographic area (in this case the UK) and crop (oilseed rape), the first task is to identify and characterise the possible recipient species. The aim here is to identify the highest-ranking and most common candidates (i.e. those most likely to form hybrids) so that these can be evaluated first for hybrid frequency. Having identified the candidate recipient species with highest likelihood of hybrid formation, the next step is to estimate total hybrid numbers across the entire country. For this, it is vital to take cognisance of the context in which hybridisation is expected. Interspecific hybrids most commonly form at low frequen-

cies, with greatest numbers expected where both species are in extremely close proximity to each other. It follows that empirical estimates of hybridisation rates should therefore be taken at sites where crop and recipient populations are sympatric (i.e. co-occur) and efforts made to determine the number of such sites within the target area. Combination of both elements will allow for a prediction of the total number of interspecific hybrids that are formed locally. Long-range hybridisation should then be predicted. This variable is more difficult to quantify and will almost invariably involve a substantial modelling component. Finally, the estimates of local and long-range hybrids should be combined to provide an overall value for hybrid abundance within the geographic area (in this case, the UK). Thus, for most cases, the process of local hybrid quantification from a given GM crop in a particular country will comprise five steps:

1 Identify and rank recipients
2 Quantify local hybrid frequency during sympatry
3 Determine the number of sympatric sites
4 Estimate long-range hybrid numbers using a modelling approach
5 Combine estimates to describe number and distribution of hybrids.

POSSIBLE RECIPIENTS OF TRANSGENES FROM GM OILSEED RAPE IN THE UK

Thankfully, the UK has one of the most extensively surveyed floras in the world (e.g. Stace 1997) and has even been surveyed for the presence and distribution of interspecific hybrids (Stace 1975). The existence of these and similar publications describing the outcomes of interspecific crosses performed as part of plant breeding initiatives (e.g. Chevre *et al.* 1997), allowed Scheffler & Dale (1994) to review the possibility of hybridisation between cultivated *B. napus* and its wild relatives in the UK. The authors named four wild species where spontaneous interspecific hybridisation had been reported with the crop (*Brassica rapa*, *B. juncea*, *B. adpressa* and *Raphanus raphanistrum*) and a further thirteen species where hybrids are formed when pollination is carried out manually. These were ranked according to the ease of hybrid formation, with the progenitor species *B. rapa* being ranked as most likely to generate hybrids. This assertion, coupled with the fact that *B. rapa* is widespread in Britain (Preston *et al.* 2002), means that effort should first centre on characterising exposure in this species before progressing to others.

LOCAL F_1 HYBRID FORMATION BETWEEN OILSEED RAPE AND *BRASSICA RAPA*

The process of estimating the frequency of local interspecific hybrids between oilseed rape and *B. rapa* is a complex one and comprises several parts. First, the rate of hybrid formation must be calculated for the various contexts in which crop and recipient come into contact with each other. Second, it is important to describe the distribution of both crop and wild relative such that the location and abundance of sympatry can be estimated. Finally, total numbers of hybrids are calculated from the number of sites of sympatry, coupled with the mean number of hybrids expected in each site annually. These aspects are examined separately below.

FREQUENCY OF HYBRIDS AT SITES OF SYMPATRY

The difference in ploidy level between the tetraploid oilseed rape (*B. napus*, 2n=38) and the diploid *B. rapa* (2n=20) means that hybrids between them are triploid (2n=3x=29) and can be readily identified by a combination of flow cytometry (to establish ploidy level) and microsatellite analysis (to distinguish hybrids from autotriploid *B. rapa*). Before attempting to quantify local hybrid abundance, however, it is important to consider the context in which crop and recipient are coincident. For instance, when surveying weedy populations of *B. rapa* for hybrids, it should be borne in mind that hybrid seed frequency may not equate to the abundance of flowering hybrids in the field. This is because B. rapa is effectively controlled as a weed during the predominant cereal phases of the rotation and hybrid seed dormancy is notably less pronounced than that of the weedy *B. rapa* (Linder 1998). It is also important to note that the density of such populations is generally low in comparison to that of the surrounding crop plants and, since *B. rapa* is self-incompatible, the flowers of many isolated *B. rapa* plants will tend to be swamped by pollen from the surrounding crop plants. Thus, hybrid seed frequency is expected to be very high in this context, even though at least some of the hybrid seeds will be removed at harvest. Several authors have estimated the frequency of hybrid seeds formed in this environment using seed-collected from *B. rapa* weeds (e.g. Jørgensen & Andersen 1994; Metz *et al.* 1997) and, unsurprisingly, recorded very high estimated hybrid seed frequencies. Jørgensen & Andersen (1994), for instance, recorded rates of 9-93% from different *B. rapa* plants in a field of oilseed rape, with a mean across all plants of 60%. As stated above, however, these rates of hybrid seed set do not necessarily relate to the abundance of flowering hybrid plants. Indeed, Wilkinson *et al.* (2003) provided the only existing estimate of hybrid plant abundance amongst five weedy *B. rapa* populations in the UK, and recorded a much lower mean frequency of just 1.9% hybrid plants (46/2388 plants screened). The difference is almost certainly attributable to the lack of dormancy of the hybrids causing most hybrids to germinate in wheat in the following year, when they are subject to effective control.

Hybrid frequency in wild, riverbank populations of *B. rapa* is similarly influenced by context. Here, it important to note that there is physical separation of the crop from the recipient plants (albeit a few meters) and that individual *B. rapa* plants are surrounded by conspecific individuals rather than by oilseed rape. For these reasons, hybrid seed and hybrid plant frequencies are both expected to be low. Scott & Wilkinson (1998) provided the first such estimate for hybrid seed, when they reported rates of 0.5 to 1.5% hybrid seed set from two sympatric populations. This compares well with the 1.5% (47/3230 plants) hybrid plant frequency recorded among eight sympatric populations of riverside *B. rapa* in the later work by Wilkinson *et al.* (2003). Thus, whilst rotation and weed control causes a significant difference in hybrid seed versus plant estimates for weedy *B. rapa*, this does not seem to be the case for the wild, riverbank populations. Clearly then, it is important to consider the two ecotypes separately when assembling an estimate of *B. napus-B. rapa* hybrid plants across a wide geographic region.

DESCRIBING THE DISTRIBUTION OF CROP – WILD RECIPIENT SYMPATRY

The task of determining how often and where oilseed rape and *B. rapa* grow together can be broken down into three components:

1 Locate oilseed rape fields
2 Locate *B. rapa* populations
3 Combine distributions to map sympatry

Locate oilseed rape. Whilst the locations of oilseed rape fields changes on an annual basis because of crop rotation, the large size of fields and the fact that oilseed rape has distinctive spectral profiles during flowering, means that it is possible to locate the precise position of such fields using remote sensing technology (Davenport *et al.* 2000). Furthermore, the availability of satellite images over many years, means that annual fluctuations in field location and crop acreage can be accommodated. Wilkinson *et al.* (2000) used this approach to identify the locations of oilseed rape fields in 1998 across an area of covering much of SE England. In order to focus attention on those fields where sympatry is possible with riverside *B. rapa*, however, the authors transferred the locations of these fields onto conventional 1:50 000 scale ordnance survey maps. A more automated strategy was adopted in later studies, where riverside oilseed rape fields were identified using overlays of digitised waterways data onto annotated satellite images (Wilkinson *et al.* 2003; Elliott *et al.* 2004).

Locate *B. rapa*. There are two distinct ecotypes of *B. rapa*; the weedy ecotype and the waterside ecotype.

Wilkinson *et al.* (2003) employed a combined strategy for determining the distribution of weedy *B. rapa* populations in which reports from agricultural consultant networks was combined with those of the wildlife UK land census (the CEH countryside Survey 2000), and supplemented with directed field surveys in regions of highest infestation. This work revealed that *B. rapa* is a comparatively rare weed, largely restricted to one region of high incidence in North Humberside.

It is rather more important to describe the distribution of the wild ecotype of *B. rapa*, partly because its increased abundance means that there should be greater opportunity for hybrid formation, and partly because there is greater scope for ecological harm. The most logical first step in the process is to identify which waterways contain *B. rapa* populations, an objective that was achieved relatively easily by reference to 82 local Floras and 601 herbarium specimens. The rather more difficult task was to describe the distribution of individuals along these waterways. This required detailed foot surveys over 300km of eight rivers and four canals representative of the catchments containing the species before the distribution profiles of *B. rapa* along rivers and canals could be modelled (Elliott *et al.* 2004). These distributions were then combined with that of oilseed rape to provide a spatially explicit estimate of the location and numbers of sympatric sites between oilseed rape and wild *B. rapa* (Wilkinson *et al.* 2003).

ESTIMATING THE FREQUENCY OF LONG-RANGE HYBRIDS.

Hybrids formed from long-range pollinations are more difficult to measure empirically and so must be estimated using a modelling approach. The key variables to consider are the separation between crop and recipient populations, pollen dispersal characteristics and the

relative competitiveness of oilseed rape and *B. rapa* pollen. Elliott *et al.* (2005) measured the separation of *B. rapa* plants from oilseed rape by reference to modelled *B. rapa* distributions generated from the survey work above and the oilseed rape positions inferred from remote sensing imagery. Collectively, this allowed workers to compile a separation profile between oilseed rape and recipient plants. Inference of gene flow from such data deserves careful consideration, however, since pollen from commercial oilseed rape fields can disperse pollen by wind or insect (Timmons *et al.* 1996) and the relative importance of each is still open to debate. In the absence of a clear system to allow long-range dispersal to be modelled effectively, Wilkinson *et al.* (2003) elected to model wind dispersal under the stated assumption that insect-mediated dispersal is either insignificantly different or at least no more prone to long-range delivery than wind-mediated dispersal. They modelled the relationship between pollen density and distance using the inverse power law relationship:

$$p(x) = \frac{e^{-x/l}}{C(c+x)^b}$$

where p is pollen density and d is distance (m), l is a scale for exponential loss of pollen by death and absorption, probably of the order of ≥100000 m (windspeed of 5 m/s=18km/h, 5 h half-lifetime gives around this number); C is a normalisation constant, b is the power-law exponent, c is a constant describing the distribution 'shoulder' and x is the distance from the field (m). Combination of isolation profile data with the airborne pollen decay relationship allowed long-range wind-borne pollen delivery to *B. rapa* populations to be estimated. Use of this value to calculate hybrid seed set relies on the relative competitiveness of oilseed rape pollen. This was estimated empirically using hybrid frequencies observed over short distances where pollen densities were known.

SPATIALLY EXPLICIT PREDICTIONS OF HYBRID ABUNDANCE

The final part in the process of amassing a national scale model of hybrid abundance and distribution is to assemble the distribution profiles of both species, together with hybridization rates calculated for each isolation distance, to compile a spatially explicit prediction of hybrid abundance. Wilkinson *et al.* (2003) combined data in this way to produce the first estimate of this kind for gene flow from *Brassica napus* to *B. rapa* in the United Kingdom. They calculated that approximately 48 000 hybrids form each year in the UK between cultivated oilseed rape and *B. rapa*. However, the significance of these F_1 hybrids rests largely on their ability to survive and produce introgressed *B. rapa* plants, and also on the capacity for subsequent spread of transgenes into other populations. It is in the latter of these two areas that we will now focus.

THE SCOPE FOR TRANSGENE SPREAD AFTER HYBRIDIZATION AND INTROGRESSION

There are some key features of the distribution of waterside *B. rapa* that provides important clues over the likely nature and speed of transgene movement between *B. rapa* populations following initial hybrid formation. The first of these is that waterside *B. rapa* is markedly more abundant along rivers than along canals, with a mean of 11.4 populations over 1 km of river compared to a mean of 0.09 populations/km for canals (Wilkinson *et al.* 2003). A tentative explanation of this observation can be made by careful examination of the phenomenon at sites where canals and rivers coincide. For example, this discrepancy is even apparent in instances where canals runs parallel to the course of the feeding river or when sites

upstream and downstream of river feed points into canal systems are compared. One obvious difference between connected canals and rivers lies in the far greater propensity of the latter to flood, especially during the winter. Canals characteristically possess numerous overflow points to reduce the incidence of flooding; a feature generally lacking from natural rivers. However, navigable rivers do contain similar points of flood control immediately upstream of locks that are used for boat navigation. This is necessary to prevent flooding of the locks. However, the practice also means that comparisons can be made of *B. rapa* abundance in adjacent stretches of river where flood control is and is not evident. When this was done for 17 locks on the river Thames, all but three exhibited a much higher abundance of *B. rapa* in the 100 m downstream of the locks (where flooding is uncontrolled) compared to that seen in the 100 m upstream section (Fig. 1). Interestingly, in the exceptions, the flood control point was very close to the lock and *B. rapa* populations were upstream of this point.

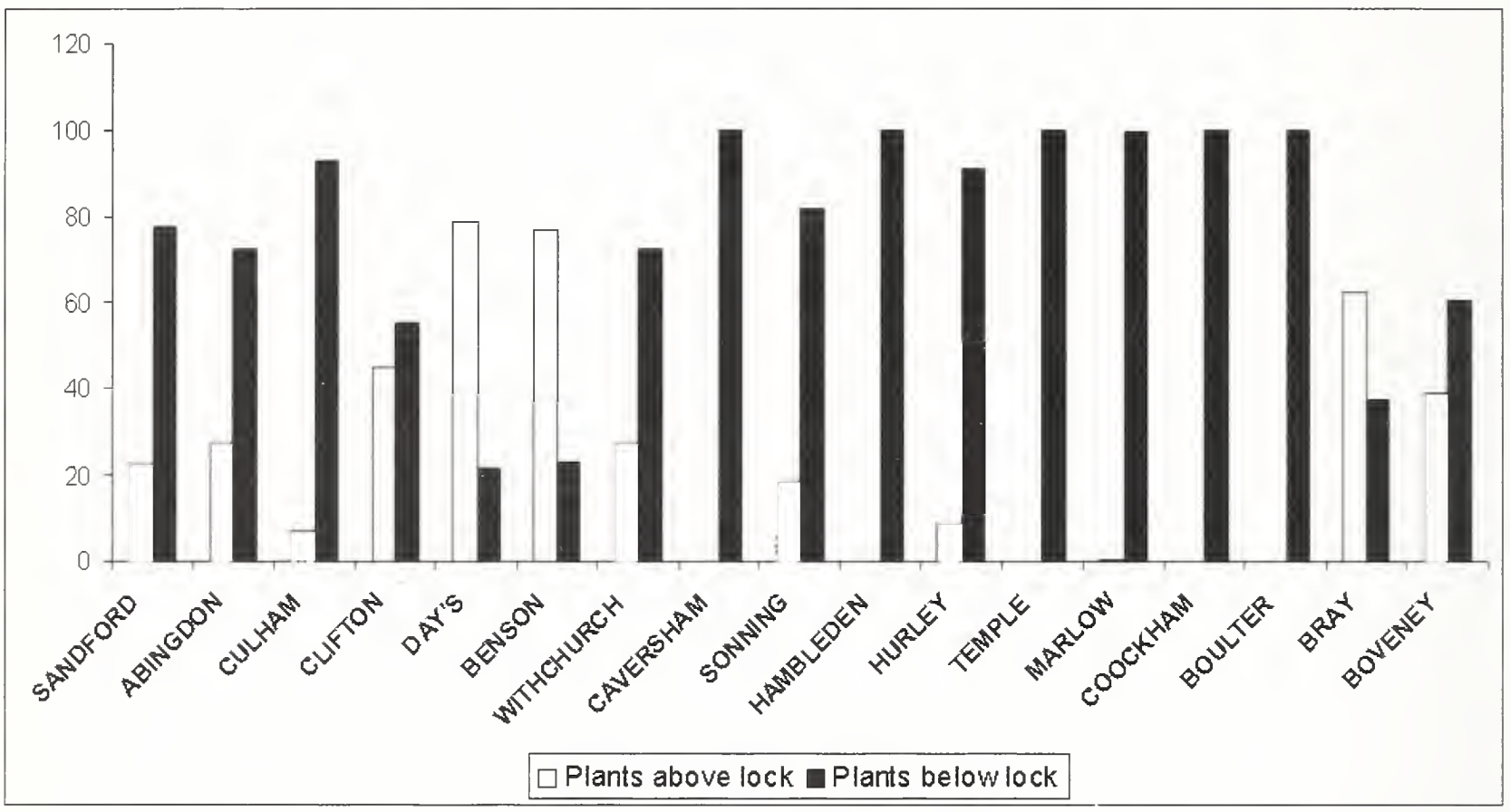

FIGURE 1. Relative abundance of *Brassica rapa* plants upstream (white) and downstream (black) of Locks along the river Thames.

Further support for a role of flooding in determining the presence of waterside *B. rapa* can be taken from the location of numerous populations that are distant from the riverbank but are nevertheless located within the limit of the mean five year flood predictions provided by DEFRA. This finding has significance as it suggests that seed dispersal may be important in effecting gene exchange between populations as well as for population establishment. To test the capacity of *B. rapa* seed to withstand long periods of submersion under natural conditions, we dredged riverbed soil from three sites along the river Thames in early spring (i.e. before *B. rapa* flowering) and overlaid the resultant slurry onto a steam-steriled compost-sand mix. The dredged soil was noted to contain a small quantity of *B. rapa* seeds, of which 5 germinated and grew into plants. Taken collectively, it seems feasible from these data that *B. rapa* can be dispersed by flooding and can retain viability even when submerged for prolonged periods. This finding could have significance in terms of predicting the pattern of secondary spread of transgenes after initial hybrid formation. This is because, unlike pollen-mediated gene movement, the dispersal of genes

by flooding would have a strong directional bias in the downstream direction. The relative importance of flood-mediated seed dispersal versus bee-mediated pollen dispersal between populations is therefore important in setting the pattern of secondary transgene spread after initial hybrid formation. This aspect is therefore currently under investigation.

A second feature of the *B. rapa* distribution that warrants attention only becomes manifest when the same stretch of river is surveyed over several years. When this was done for the river Thames between Eton and Oxford using GPS-assisted positioning of the populations identified, it was noted that the location of individual populations was not fixed but was subject to radical year-to-year movement (e.g. Fig. 2).

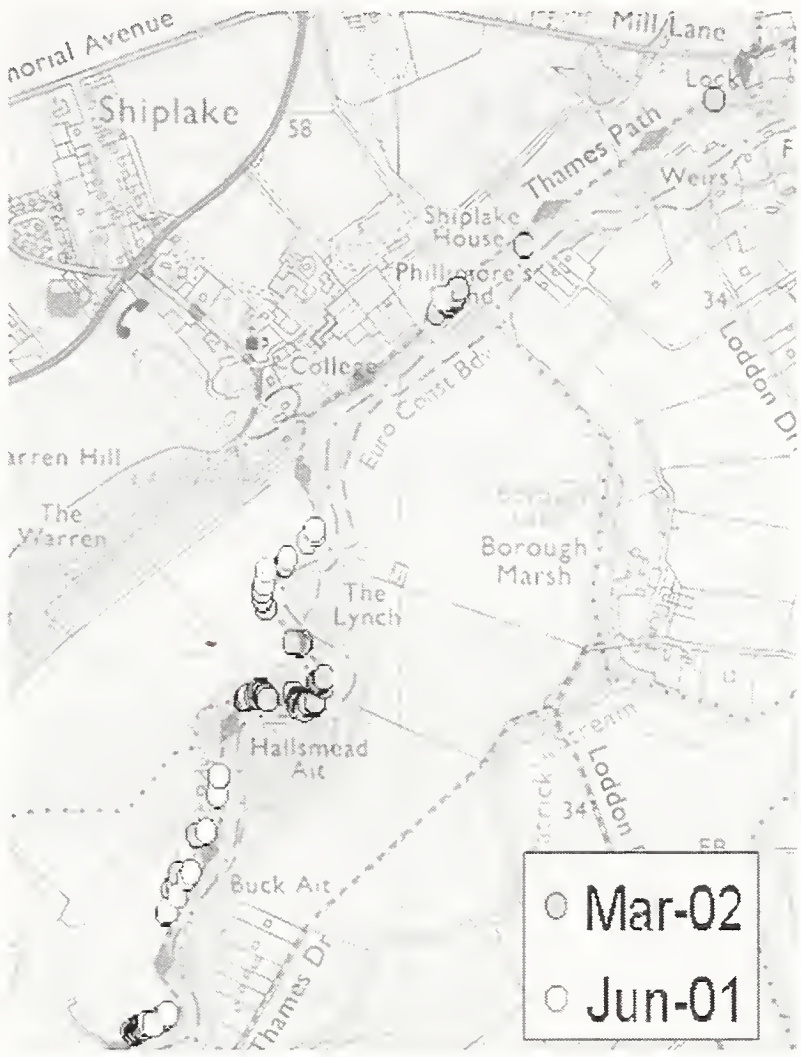

FIGURE 2. Location of *Brassica rapa* populations on the river Thames near Shiplake in 2001 (Grey circles) and 2002 (Light circles).

Furthermore, even when the precise positions of individual plants was marked in a population that reappeared in the same site (identical grid reference), it was evident that the plants occupied a different physical space to the preceding year in a manner that was independent of the direction of water flow (Fig. 3).

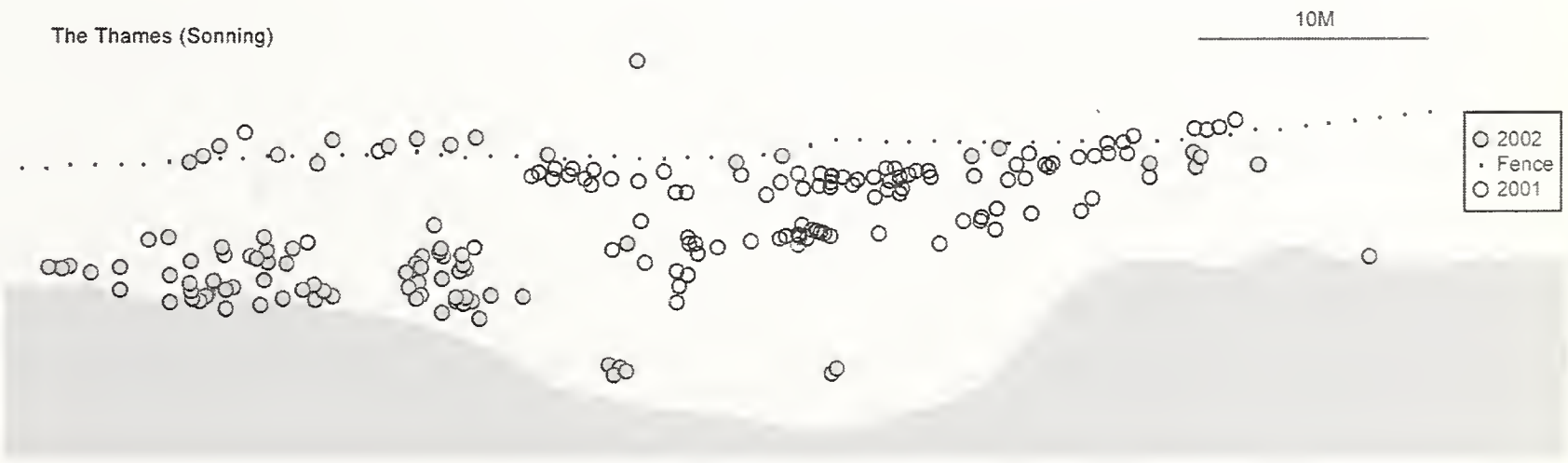

FIGURE 3. See text for details

Perhaps the most reasonable explanation of these observations is that recruitment of *B. rapa* seedlings into the flowering plant population is highly dependent upon riverbank disturbance and that the most common cause of such disturbance is flooding. We have tested the capacity of extinct sites that no longer contain *B. rapa* to yield populations by simply removing surrounding vegetation and disturbing topsoil in spring following the annual winter floods. We noted that *B. rapa* seedlings germinated and became recruited only in the disturbed quadrats, supporting the suggestion that vegetation disturbance is a necessary prerequisite for *B. rapa* establishment. Overall then, we can infer that the presence of *B. rapa* populations is highly variable between years and that flooding is probably important in creating the opportunity for recruitment from the soil seed-bank but also in enabling interpopulational gene movement. Accordingly, we are now in the process of modelling the spread of genes within and between *B. rapa* populations based on a life history that is dominated by recruitment dynamics from the seed-bank.

CONCLUSIONS

There is currently only a limited number and complexity of transgene constructs in GM cultivars, although this situation seems set to change as advances in genomics and post-genomics research radically increase the availability of new constructs. This trend sets new challenges for risk assessment research and dictates that regulators assemble as much generic data as possible. For risks relating to gene flow from GM oilseed rape in the UK, several points arising from the work described here should be considered for future submissions for commercial release.

1 Hybrids will form with *B. rapa* in scattered locations, mostly across eastern England
2 The limited number of sites of hybridization means that the extent and speed of subsequent infraspecific gene movement, together with any fitness advantage, will determine the ultimate transgene distribution
3 Infraspecific gene flow between populations is mediated by pollen and seed dispersal (flooding)
4 The directional bias of flood-mediated seed dispersal means that the site of transgene recruitment (hybridisation) will have a profound effect on its capacity to spread to other populations
5 The appearance of plant populations is partly a function of disturbance (flooding and river management). It follows that the rate of spread and fixation of any transgene will be partly influenced by the amount of disturbance.
6 These data are currently being assembled to develop a spatially explicit model of transgene spread

REFERENCES

CHEVRE, A.M., EBER, F., THIS, P., BARRET, P., TANGUY, X., BRUN, H., DELSENY, M., RENARD, M. (1997). Selection of stable *Brassica napus*, *B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*). 1. Identification of molecular markers, chromosomal and genomic origin of the introgression. *Theoretical and Applied Genetics* **95**: 1104–1111.

DANIELL, H. (1999). New tools for chloroplast genetic engineering. *Nature Biotechnology* **17**: 855–856.

DAVENPORT, I.J., WILKINSON, M.J., MASON, D.C., JONES, A.E., ALLAINGUILLAUME, J., BUTLER, H.T.& RAYBOULD, A.F. (2000). Quantifying gene movement from oilseed rape to its wild relatives using remote sensing. *International Journal of Remote Sensing* **21**: 3567–3573.

ELLIOTT, L.J., MASON D.C, WILKINSON M.J., ALLAINGUILLAUME J., NORRIS C., ALEXANDER, M., WELTERS, R. (2004). Using satellite image processing for large-scale study of gene flow from genetically modified rapeseed. *Journal of Applied Ecology* **41**: 1174–1184.

ELLIOTT, L.J., MASON, D.C., WILKINSON, M.J. & ALLAINGUILLAUME, J. (2005). The value of remote sensing technology for GM risk assessment. In: VAN EMDEN, H.F. & GRAY, A.J. (eds.) *GMOs-Ecological dimensions.* AAB, Warwick UK pp. 47–53..

FRIESEN, L.F., NELSON, A.G. & VAN ACKER. R.C. (2003). Evidence of contamination of pedigreed Canola (*Brassica napus*) seedlots in Western Canada with genetically engineered herbicide resistance traits. *Agronomy Journal* **95**: 1342-1347.

GIDDINGS, G. (2000). Modelling the spread of pollen from *Lolium perenne*. The implications for the release of wind-pollinated transgenics. *Theoretical and Applied Genetics* **100**: 971–974.

JØRGENSEN, R.B. & ANDERSEN, B. (1994). Spontaneous hybridization between oilseed rape (*Brassica napus*) and weedy *Brassica campestris* (Brassicaceae): a risk of growing genetically modified oilseed rape. *American Journal of Botany* **81**: 1620–1626.

LACK, G. (2002). Clinical risk assessment of GM foods. *Toxicology Letters* **127**: 337–340.

LINDER, C.R. (1998). Potential persistence of transgenes: Seed performance of transgenic canola and wild × canola hybrids. *Ecological Applications* **8**: 1180–1195.

LOOS, C., SEPPELT, R., MEIER-BETHKE, S., SCHIEMANN, J. & RICHTER, O. (2003). Spatially explicit modelling of transgenic maize pollen dispersal and cross-pollination. *Journal of Theoretical Biology* **225**: 241–255.

METZ, P.L.J., JACOBSEN, E., NAP, J.P., PEREIRA, A. & STIEKEMA, W.J. (1997). The impact on biosafety of the phosphinothricin-tolerance transgene in inter-specific *B. rapa* × *B. napus* hybrids and their successive backcrosses. *Theoretical and Applied Genetics* **95**: 442–450.

OLIVER, M.J., QUISENBERRY, J.E., TROLINDER, N.L.G. & KEIM, D.L. (1999). *Control of Plant Gene Expression*. US Patent no. 5,925,808.

OOMS, G., BAINS, A., BURRELL, M., KARP, A., TWELL, D. & WILCOX, E. (1985). Genetic manipulation in cultivars of oilseed rape (*Brassica napus*) using *Agrobacterium*. *Theoretical and Applied Genetics* **71**: 325–329.

PRESTON, C.D., PEARMAN, D.A. & DINES, T.D. (2002). *New Atlas of the British & Irish Flora*. Oxford University Press, Oxford.

ROSELLINI, D., PEZZOTTI, M. & VERONESI, F. (2001). Characterization of transgenic male sterility in alfalfa. *Euphytica* **118**: 313–319.

REIGER, M.A., LAMOND, M., PRESTON, C., POWLES, S.B. & ROUSH, R.T. (2002). Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* **296**: 2386–2388.

SQUIRE, G.R., BROOKS, D.R., BOHAN, D.A., CHAMPION, G.T., DANIELS, R.E., HAUGHTON, A.J., HAWES, C., HEARD, M.S., HILL, M.O., MAY, M.J., OSBORNE, J.L., PERRY, J.N., ROY, D.B., WOIWOD, I.P. & FIRBANK, L.G. (2003). On the rationale and interpretation of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of the Royal Society, London series* B **358**: 1779–1799.

SCHEFFLER, J.A. & DALE, P.J. (1994). Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. *Transgenic Research* **3**: 263–278.

SCOTT, S.E. & WILKINSON, M.J. (1998). Transgene risk is low. *Nature (London)*, **393**: 320.

SONG, K. & OSBORN, T.C. (1992). Polyphyletic origins of Brassica-napus - new evidence based on organelle and nuclear RFLP analyses. *Genome* **35**: 992–1001.

STACE, C.A. (1975). *Hybridization and the flora of the British Isles*. London: Academic Press.

STACE, C.A. (1997). *New Flora of the British Isles*, edn 2. Cambridge: Cambridge University Press.

TIMMONS, A.M., CHARTERS, Y.M., CRAWFORD, J.W., BURN, D., SCOTT, S.E., DUBBELS, S.J., WILSON, N.J., ROBERTSON, A., O'BRIEN, E.T., SQUIRE, G.R., & WILKINSON, M.J. (1996) Risks from transgenic crops. *Nature* (London) **380**: 487.

WAINES, J.G. & HEDGE, S.G. (2003). Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. *Crop Science* **43**: 451–463.

WILKINSON, M.J., DAVENPORT, I.J., CHARTERS, Y.M., JONES, A.E., ALLAINGUILLAUME, J., BUTLER, H.T., MASON, D.C. & RAYBOULD, A.F. (2000). A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. *Molecular Ecology* **9**: 983–991.

WILKINSON, M.J., ELLIOTT, L.J., ALLAINGUILLAUME, J., SHAW, M.W., NORRIS, C., WELTERS, R., ALEXANDER, M., SWEET, J. & MASON, D.C. (2003). Hybridization between *Brassica napus* and *B. rapa* on a National Scale in the United Kingdom. *Science* **302**: 457–459.

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Endemic vascular plants in the Nordic flora

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ABSTRACT

The endemic taxa of vascular plants of the Nordic countries amount to about 130, of which 46 are currently regarded as species (*Hieracium*, *Taraxacum*, and the *Ranunculus auricomus* complex excluded). A postglacial origin, i.e. a time span of maximally 10,000 years of evolution, is now postulated for all Nordic endemics. There are five allopolyploid sexual endemic species; *Arabidopsis suecica*, *Corydalis gotlandica*, *Draba cacuminum*, *Primula scandinavica* and *Saxifraga osloënsis*. Outside the large apomictic groups mentioned above, only about 40 apomictic endemics are recognized. A large proportion of the non-hybrid and non-apomictic endemics have probably originated from ecotypes. Many Nordic endemics are members of intricate species complexes with subtle and often disputed taxonomic limits. The Nordic endemics are geographically concentrated, but far from exclusively, to a number of 'hot' areas with particular ecological and historical conditions, the most important of which are the Scandes, the Baltic land-lift shores and the Baltic limestone islands of Öland and Gotland. A smaller number of endemic taxa are found at the coasts of NW Jylland (Denmark), along the Arctic Ocean coast, and in Iceland. The isolated Nordic islands and the island groups to the north and west (Spitzbergen, Jan Mayen, and the Faroes) have few or no endemic taxa.

Keywords: endemism, Scandinavia, North Atlantic, Scotland, glaciation.

INTRODUCTION

There are few endemic taxa in the Nordic vascular flora (as long as apomictic groups are disregarded), and their taxonomic rank is usually low. The latest glaciation in Norden terminated about 10,000 years ago. With present evolutionary models that time span is considered long enough to allow for the amount of differentiation by ecological and geographical isolation, though many endemics appear to be much younger. There are no indications for the previously much-favoured view that Nordic endemics should emanate from glacial survivors. For more comprehensive discussions of endemism in Norden see Borgen (1987), Brochmann *et al.* (2003), Dahl (1989), Jonsell (1988, 1990a, 1990b, 1997), Jonsell & Karlsson (2004).

The area considered here is that covered by *Flora Nordica* (Jonsell 2000, 2001). Some consideration is also given to the area between the Finnish-Norwegian border and the White Sea, i.e. the Kola Peninsula and Russian Karelia. This area is geologically a part of the Fennoscandian shield and viewed from the point of natural history it is connected to Fennoscandia. With microspecies of the three major groups of apomicts excluded the

number of endemics at species, subspecies and varietal level in the *Flora Nordica* area is *c.*130. By including northwestern Russia the number is increased to about 160, and if the whole Baltic area (i.e. the southeastern and southern coasts) is included, the number reaches 180. Inclusion of the whole North Atlantic area, i.e. Greenland and the British Isles as well, would add between 25 and 30 taxa.

In comparisons with the endemism of other regions it is important that they are made on similar taxonomic levels, and take into account the breeding systems. The origins of endemism are multifarious and often poorly understood, even within each region quite different evolutionary situations may have led to endemism. According to the mode of origin many, but far from all, Nordic endemics can be grouped into the three categories discussed in detail below.

The taxa considered here are comparatively readily distinguishable, but their taxonomic status is not always quite clear. Some of them belong to aggregates not sufficiently analysed even within Norden and it is perhaps not possible to maintain them all as separate taxa. Some may have a wider distribution than presently assumed and occur in Russia, between the White Sea and northern Urals and even further east.

Another question is how to define 'endemic' taxonomically. The number of endemics found in Norden will depend very much on whether or not we count the endemic apomictic taxa. Some apomictic groups, e.g. *Rubus*, *Sorbus* and *Alchemilla*, have a moderate number of distinct agamospecies with local, regional or even wider distribution; they are mentioned here as equivalents to non-apomictic taxa. There are also large apomictic groups, with hundreds of described 'microspecies' and still many undescribed ones, viz. the *Ranunculus auricomus* group and the genera *Taraxacum* and *Hieracium*. They are excluded here mainly because the distribution of their species is poorly known outside Norden. The apomictic groups are enumerated below.

Some of the more distinct endemics probably originated from ecotypes. By definition (Turesson 1925) these may have polytopic origins; most cases are poorly analysed. Few ecotypes have been formally described.

ENDEMIC ALLOPOLYPLOIDS

Only a few of the endemic sexual taxa can undoubtedly be ranked as species. Among those that can, five are the results of allopolyploidy, an abrupt mode of speciation on an evolutionary time scale:

Corydalis gotlandica (2n = *c.*30) is known from a few localities in the western part of the Baltic island of Gotland. It grows, and is expanding, in a small scale agricultural landscape and on an open limestone island some five kilometres off the coast. Its distribution and behaviour suggest a comparatively recent origin. The parental species are *C. intermedia* (2n = 16) and *C. solida* (2n = 16) (Lidén 1991), which are both present on Gotland.

Arabidopsis suecica (2n = 26) (Colour Plate 2a). This species is widely distributed on disturbed ground in Sweden and Finland, particularly on gravelly roadsides and railway areas, and not in anything like natural habitats. Its possible origin is in southeastern Finland with its vast glaciofluvial gravelly moraines, which in early postglacial times offered natural open gravelly areas free from competition. The parental species are *A. arenosa* (2n = 16, 32) and *A. thaliana* (2n = 10) (O'Kane *et al.* 1996). They are both common within much of the area of *A. suecica*.

Draba cacuminum (2n = 64) is a rare high mountain species with different races in two separate areas of the Scandes: the south Norwegian mountains from N Buskerud to Sør Trøndelag (subsp. *cacuminum*), and further north in Nordland and adjacent parts of Sweden from Lycksele to Lule Lappmark (subsp. *angusticarpa*). The species has a multiple origin with the common *D. norvegica* (2n = 48) as one parental species and probably the much rarer *D. fladnizensis* (2n = 16) (Brochmann & Elven 1992) as the other.

Saxifraga osloënsis (2n = 44) (Colour Plate 2b). This species is confined to base-rich outcrops in a zone across middle Scandinavia from the Oslo region in Norway to Uppland in E Sweden. It probably originated in early post-glacial time from the montane, northern *S. adscendens* (2n = 22) and the southern, calcicole *S. tridactylites* (2n = 22) (Knaben 1954, Brochmann *et al.* 1996). The parental species are still widespread to the north and the south, respectively, of the zone of *S. osloënsis*, but the three species are not known to grow on the same spot anywhere.

Primula scandinavica (2n = 72). This species is widespread in more oceanic parts of the Scandes from Norway (Rogaland) and W. Jämtland in Sweden north to western Lapponia tornensis in Sweden and Troms in Norway. According to Knaben (1982) it probably originated from *P. farinosa* (2n = 18, 36), nearest in C Sweden, and *P. scotica* (2n = 54), now only in Scotland. Today the parental species are far apart but Knaben speculated that they might have been in contact in glacial and early late-glacial times on the North Sea Continent, which was apparently of great importance for glacial survival and as a source of immigrants in late glacial time. Other interpretations are, however, possible (Richards 1993).

HYBRIDS

There are various asexual ways for hybrids to reproduce and spread more or less independently of their parents. Four well defined taxa of this kind are among the Nordic endemics.

Salix ×arctogena is basically a triple hybrid between *S. herbacea*, *S. polaris* and *S. phylicifolia*, but in some localities there is evidence that *S. glauca* and *S. lapponum* also participate. *S. ×arctogena* is pollen and seed fertile; it has arisen polytopically in at least seven areas in the Scandes (Elven in Jonsell 2000).

Saxifraga ×opdalensis is a hybrid between *S. cernua* and *S. rivularis* with one local occurrence in south central Norway (the Dovre mountains). It does not set seed but reproduces by means of bulbils. Further populations, probably of the same parentage, are known from some places in northern Norway and in Swedish Lapland. A similar plant, described as '*S. ×svalbardensis*', has arisen in Svalbard from a cross between Svalbard forms of the same two species (Steen *et al.* 2000). All these plants are here included in *S. ×opdalensis*.

Poa ×herjedalica is the viviparous hybrid between *P. alpina* and *P. pratensis* subsp. *alpigena*. It has a wide distribution throughout the Scandes and has almost certainly arisen polytopically.

Carex halophila is a member of the *C. recta* complex, an amphi-Atlantic aggregate of hybrids between, on the one hand, *C. paleacea* and *C. salina*, and on the other hand, *C. acuta*, *C. aquatilis* and *C. nigra*. *C. halophila* occurs along the coasts of northern Norway and the Kola Peninsula, and, in deviating forms, in the Gulf of Bothnia.

APOMICTS

Agamospermy is another process that leads to rapid establishment of new taxa. There are 13 genera with agamospermous endemics in Norden. This is by far the largest element of the Nordic endemics. The three largest agamospermous groups, viz. the *Ranunculus auricomus* group and the genera *Hieracium* and *Taraxacum,* comprise the great majority of taxa.

The Ranunculus auricomus group. 605 Nordic species have been described, and most of them are only known from Norden. The treatment in *Flora Nordica* includes a number of examples and a checklist to all known Nordic taxa (Ericsson in Jonsell 2001). In all likelihood, very many taxa remain to be described.

Alchemilla is represented in Norden by 22 indigenous or archaeophytic species, most of them with a rather wide distribution. Only three Nordic endemics, *A. faeroënsis* (in the Faroes and E. Iceland), *A. semidivisa* (both of the *Splendentes* group and intermediate to *A. alpina*) and *A. taernaënsis*, have been described; a further two, *A. borealis* and *A. oxyodonta*, have but little of their range outside Norden. There is also a moderate number of undescribed (and partly insufficiently studied), mostly very local taxa.

Cotoneaster. There are four indigenous Nordic species of this largely agamospermous genus. Three of them occur also in the Baltic states; the fourth, *C. kullensis*, is a local taxon from southernmost Sweden.

Potentilla. Numerous agamospermous species have been described within this genus, but only two Nordic endemics are known, viz. *P. insularis* from Svalbard and *P. sterneri* from coastal SE Sweden.

Rubus subgen. *Rubus*. Of the 98 accepted spontaneous taxa of brambles known from Norden only 11 are Nordic endemics. All except one belong to sect. *Corylifolii*, which comprises hybrid derivatives probably of fairly recent origin. Some further local taxa are known, and some of them have been validly described, but bramble biotypes with a very limited distribution are not taxonomically accepted nowadays (Weber 1972).

Sorbus. In addition to the three diploid sexual species (2n = 34) there are nine currently accepted, described apomictic triploids (2n = 51) and tetraploids (2n = 68). One of these (*S. aria*) is fairly widespread in Europe. One (*S. intermedia*) is present in Norden and Estonia only, the occurrence in Scotland being a recent introduction. The other seven are endemic to Norden. *S. hybrida* and *S. meinichii* have comparatively wide distributions (southern Norway, coastal southern Sweden), while the other five are restricted to fairly small areas in the west. The genus is under revision and the number of described endemics may possibly increase.

Hieracium. Within *Hieracium* more than 5000 presumably apomictic species have been described from Norden. The native species are arranged into 14 sections with tens to hundreds of species, some mainly occurring in the man-made landscape, others montane or alpine. In all likelihood most of the species are endemic to Norden. The *Hieracium* flora is well investigated in large parts of Norden, but in and near the Scandes numerous species probably remain to be described.

Pilosella. This genus is notoriously difficult due to the presence of facultative agamospermy, leading to the formation of numerous microspecies whose distinctness is partly concealed by more continuous variation among the sexual forms. A new taxonomic system,

which seems to work well at least within Norden, was recently proposed by Tyler (2001). He distinguished 16 indigenous taxa, of which three are endemic.

Taraxacum. The genus comprises about 1000 described species in Norden distributed among 14 sections, including between two and hundreds of species. The rate of endemism varies greatly among sections and between habitats, being relatively high in alpine and montane areas, fairly low in the man-made landscape. The smallest section includes *T. dovrense*, endemic in the S. Norwegian mountains and closely related to the circumarctic *T. arcticum*. A limited number of yet undescribed species have been identified.

Gymnadenia. This genus comprises four species, of which two are agamospermous and endemic to Norden. *G.* (*Nigritella*) *nigra* subsp. *nigra* is a triploid (2n = 60) with related sexual taxa in Central Europe, whereas *G.* (*Gymnigritella*) *runei* is a tetraploid (2n = 80) which has arisen locally with *G. nigra* subsp. *nigra* as one parent and *G. conopsea* (2n = 40) as the other (Teppner & Klein 1989, Hedrén 1999).

Calamagrostis. This grass genus comprises seven Nordic species, three of which are agamospermous. One of these, *C. chalybaea*, is endemic. It is widespread in northern Sweden and known also from Nord-Trøndelag and Nordland in Norway as well as from one locality in the Kola Peninsula. Its closest relative appears to be the sexual *C. obtusata* of eastern Russia.

Hierochloë. As currently understood (Weimarck 1971, 1986) the genus comprises seven taxa in Norden, all except one (*H. australis*) at least facultatively apomictic. Most taxa have fairly wide to very wide distributions, but *H. odorata* subsp. *baltica* appears to be endemic to Norden and Estonia. It probably developed from the amphi-Atlantic subsp. *odorata*.

Poa. Apomixis is widespread in this genus, but within Norden, agamospermous endemics have been described only within *P. arctica* (Nannfeldt 1940). In the southern Scandes there are three fairly well-delimited races, one of which (subsp. *stricta*) is viviparous. In the northern Scandes the variation is more continuous and only two very local taxa have been possible to discern (apart from subsp. *caespitans*, which has a wide amphi-Atlantic distribution).

AREAS OF ENDEMISM AND EXAMPLES OF TAXA

There are three major areas of endemism in Norden. Apparently, these areas have, or have had, ecological conditions promoting evolution. The areas are presented below with a few examples of their endemics. In addition the Arctic coast of Norway extending into the Russian Kola Peninsula, coastal dunes and sea-facing hillsides in northwestern Jylland in Denmark, and Iceland each house a few endemic taxa (see further Jonsell & Karlsson 2004). The island groups of Svalbard and the Faroes have only one or two endemics each, and the very isolated, volcanic island of Jan Mayen none. On the other hand some 60 endemics at all taxonomic levels have distributions not coinciding with any others, among those the allopolyploid *Saxifraga osloënsis*.

The Scandes and adjacent areas

The majority of the endemics occur in the low-alpine belt, and some extend also to the birch belt below and to the mid-alpine above (Jonsell 1990 b). Few taxa are exclusive to any of the latter belts. Out of the total of 180 endemics in Norden and adjacent areas, 47 taxa belong to this group. Of these, nearly all are known from Norway, which has the core of the Scandinavian mountain ridge. The number of taxa declines eastwards (27 in Sweden, 15 in Finland, 11 in northwestern Russia).

Most of these endemics are currently evaluated as subspecies, whilst six are regarded as varieties. Three taxa are hybrids (*Salix ×arctogena*, *Saxifraga ×opdalensis* and *Poa ×herjedalica*). Of the nine species two are allopolyploids (*Draba cacuminum* and *Primula scandinavica*), whereas four are agamospermous (*Alchemilla semidivisa*, *A. taernaënsis*, *Taraxacum dovrense* and *Gymnadenia runei*).

Among the few sexual endemics currently evaluated as species are *Silene wahlbergella,* a pronounced selfer within the intricate arctic *S. uralensis* group, as well as *Euphrasia hyperborea* and *Antennaria nordhageniana,* both members of complexes, in which the differences between species are disputed.

Isolation in various mountain massifs leading to vicariant endemics in a species or species group is of little significance. *Papaver radicatum* with its many local or regional subspecies in the Scandes is much cited in Norden as an example, even held to indicate a history of glacial survival. It is now rather thought that the degree of differentiation between most of the *P. radicatum* subspecies hardly surpasses that found within more or less coherent Arctic populations (Solstad *et al.* 1999, 2003). This is also true of subsp. *laestadianum*, which is an octoploid (2n = 56) in contrast to all other Nordic *P. radicatum*, which are decaploid (2n = 70).

Öland and Gotland

In Öland and Gotland open habitats on limestone pavements (alvar plains) play a major role and in Gotland semi-open calcareous pine forest is still common. These and other habitats on the two islands harbour a rich flora which comprises 20 taxa endemic to Norden or nearly so. Five of these are known only from Öland (*Helianthemum oelandicum* var. *oelandicum* and var. *canescens*, *Galium oelandicum*, *Artemisia oelandica* and *Crepis tectorum* subsp. *pumila*), four only from Gotland (*Pulsatilla vulgaris* subsp. *gotlandica*, *Corydalis gotlandica*, *Euphrasia salisburgensis* var. *schoenicola* and *Crepis tectorum* var. *glabrescens*). *Arenaria gothica* var. *gothica* has one occurrence outside Gotland, see below. The remaining ten taxa are known from both Öland and Gotland, two of them (*Cotoneaster canescens* and *Artemisia maritima* subsp. *humifusa*) also from Estonia.

The majority of the Baltic island endemics are classified as varieties or subspecies. Among the five species the allopolyploid *Corydalis gotlandica* was presented above. *Coton-easter canescens* and *Pilosella dichotoma* are agamospermous apomicts. *Galium oelandicum* and *Artemisia oelandica* are sexual and not evidently hybridogenous; the former is a member of the *G. pumilum* complex where the limits between the often rather narrowly endemic species are subtle. *A. oelandica* is only doubtfully distinct from the polymorphic *A. laciniata* of eastern Europe and western Asia. The spectacular *Helianthemum oelandicum* with its two endemic races on Öland is conspecific with taxa in western and central Europe, the Caucasus and Turkey.

Not all endemics occur in the various alvar habitats. *Euphrasia salisburgensis* var. *schoenicola* grows in spring fens, *Artemisia maritima* subsp. *humifusa* on seashores, and *Corydalis gotlandica* and *Euphrasia stricta* var. *suecica* belong to the man-made landscape. Several of the endemics appear to be relics from a much wider range in Scandinavia and other parts of Europe in late-glacial or early post-glacial times. Among those are *Arenaria gothica,* which is widespread on calcareous gravel in Gotland and endemic to that island but for a local disjunct population in a similar habitat in Mt Kinnekulle, Västergöt-

land, Sweden. It seems to be a relic from the early post-glacial, differentiated within the *A. ciliata* complex, which shows many disjunctions in NW Europe. Material referred to as *A. gothica* var. *fugax* from Swiss Jura remains to be critically reassessed.

Possibly the isolation between Öland and Gotland has promoted differentiation in some taxa. There are clear morphological differences between the islands within both *Euphrasia stricta* var. *gotlandica* (Karlsson 1986) and *Pilosella cymosa* var. *gottlandica* (Tyler 2001), and the local dwarf variants of *Crepis tectorum* are currently regarded as different taxa. However, parallel evolution of variants within these taxa on the two islands is also possible; in *Crepis tectorum* the latter hypothesis is the most likely according to Andersson (1990).

The Baltic shores

Numerous endemics are bound to the shores of the Baltic. In its northern parts (the Gulf of Bothnia, the Gulf of Finland and the northern part of the Baltic proper) there is still land uplift of up to ca 80 cm in a century on very flat shores, which means that large areas of land arise (Fig. 1). Therefore a permanent pioneer situation prevails on these shores, which is supposed to promote evolution and establishment of new taxa (Jonsell 1988, 1990a). The isolation of populations of wide-spread species on the coasts of the brackish, non-tidal Baltic has also been important. Flooding during storms contributes to make the habitats extremely unstable. The salinity gradient along the Bothnian coast also influences the distribution of the constituent species.

Fourteen endemic taxa are restricted to the northern part of the Baltic as defined above, three of which are generally treated at species level: *Alisma wahlenbergii*, *Deschampsia bottnica* (Colour Plate 2c) and *Euphrasia bottnica*. *Alisma wahlenbergii* occurs now only in the innermost part of the Bothnian Gulf and the former bay of the Baltic which is now Lake Mälaren (W of Stockholm). It appears to be a brackish-water derivative of the widespread freshwater species *A. gramineum* (Jacobsson 2003). *Deschampsia bottnica* (although morphologically quite distinct) belongs to the *D. cespitosa* complex and has close affinities to taxa on the Arctic coasts of N Russia, Siberia and in Beringia. *Euphrasia bottnica* on the contrary is a very distinct taxon for its genus, endemic to the shores of the Bothnian Gulf in Sweden and Finland, with its closest relatives in N America. Among the hemiparasites there are two more endemics, but less pronounced, in *Euphrasia* and in *Odontites*.

Three more taxa, *Primula nutans* subsp. *finmarchica*, *Sonchus arvensis* var. *maritimus* and *Carex halophila*, occur also along the Arctic coast of Norway and northwestern Russia. The local variant of *C. halophila* in the northern Baltic appears, however, to be distinct from the variants on the Arctic coast. *P. nutans* is represented by var. *jokelae* in the northern Baltic (and the White Sea area), whereas var. *finmarchica* grows on the Arctic coast.

Eight taxa have a more southern distribution in the Baltic and thus have part of their distribution area on shores which are nowadays affected by the land uplift to a somewhat lower degree (about 40 cm in a century on less flat shores). The most clear-cut of these are the almost glabrous *Mentha aquatica* var. *litoralis* and the narrow-leaved *Veronica longifolia* var. *maritima*.

Finally, five taxa are found in the southern Baltic and on the western coasts of southern Scandinavia, viz. *Polygonum oxyspermum*, *Cakile maritima* subsp. *baltica*, *Lotus corniculatus* var. *alandicus* and var. *carnosus* and *Cuscuta europaea* subsp. *halophyta*. Despite their deviating distribution their origin is probably in some way connected to the history of the Baltic and they are best treated in this context. *Polygonum oxyspermum* is closely related to

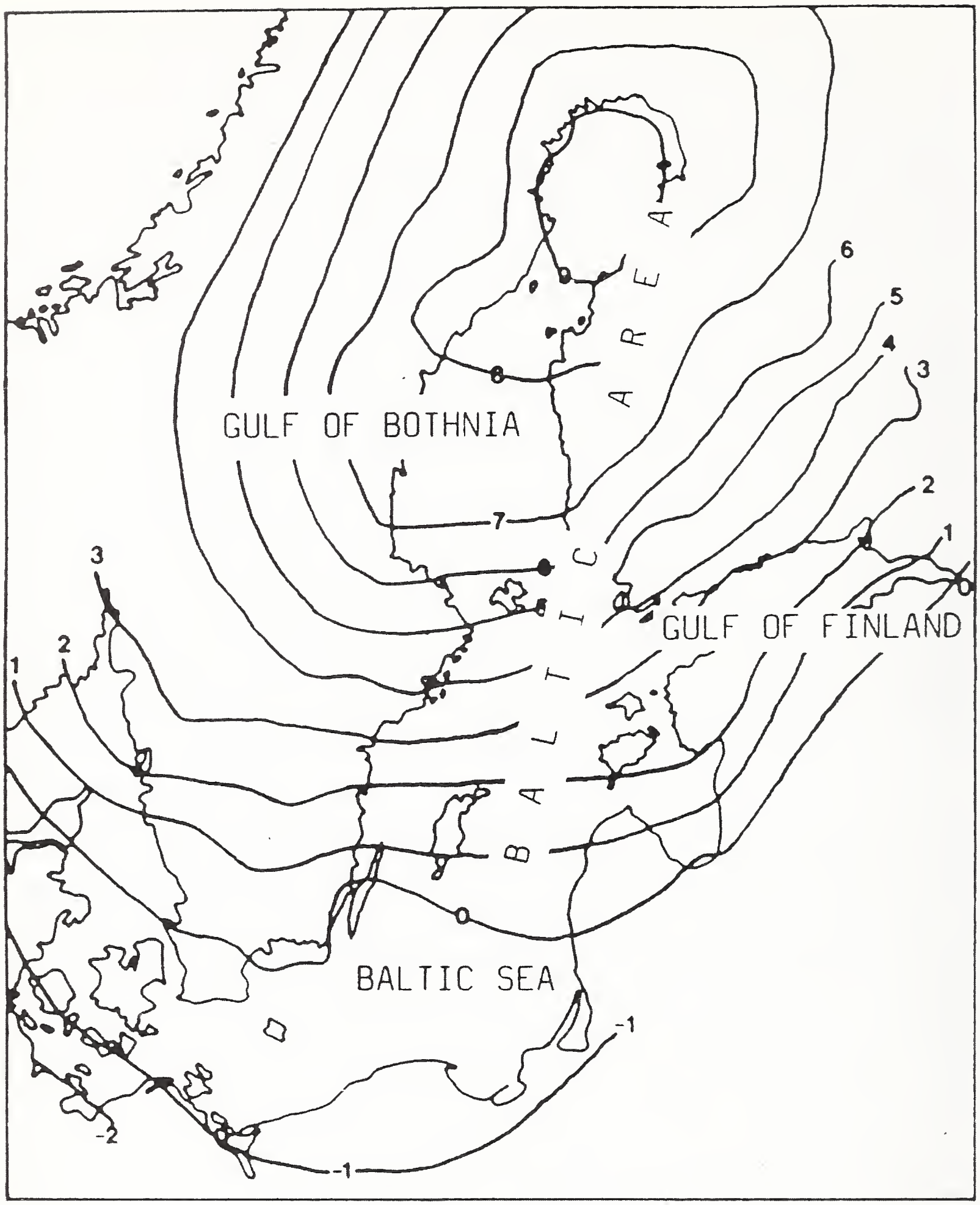

FIGURE 1. Isopleths of the present-day land uplift in the Baltic area im mm/year. The values are relative, *i.e.* not corrected for the eustatic rise in sea-level (*c.*1mm/year). From Ericson & Wallentinus (1979).

P. raii of the Atlantic coasts. It has probably developed as a consequence of isolation on brackish, non-tidal Baltic shores.

All in all, there are 30 endemic seashore taxa associated with the Baltic and present in Norden. All of these occur in Finland; in Sweden all are known except three Gulf of Finland endemics (*Silene vulgaris* var. *littoralis*, *Lotus corniculatus* var. *maritimus* and *Odontites littoralis* subsp. *fennicus*). About half the number (14 taxa) are found in the Baltic outside Norden.

CONNECTIONS TO SCOTLAND

The immigration of taxa which have evolved into endemics in Norden is multifarious. In this context only connections to the British Isles and its glacial surroundings, the North Sea Continent will be considered. The somewhat enigmatic origin of *Primula scandinavica* was mentioned earlier. Other examples relate to the famous taxa restricted to special habitats in Yorkshire, including the Teesdale limestone. Among these are *Viola rupestris*, the British populations of which have obvious isoenzymatic similarities with *V. rupestris* subsp. *relicta*, an endemic of the Scandes on limestone from W Central Norway northwards (Jonsell *et al.* 2000). Other examples probably recruited from Britain are to be found in *Cochlearia officinalis*, which has two endemic subspecies in Norway, and *Salix caprea* subsp. *sphacelata* in most of the Scandes at low levels.

If the geographical scope is extended to include Scotland a few more endemics can be added:

Arenaria norvegica subsp. *norvegica,* common in Iceland, scattered along the Scandes and extremely local in north-western Scotland and western Ireland. In Yorkshire it is represented by a different subspecies, subsp. *anglica,* which links this species complex, often called the *A. ciliata* complex, to that important area of relict distributions. The species is accordingly a North Atlantic endemic. *Arenaria pseudofrigida,* is an Arctic – North Atlantic endemic of the same complex, which reaches W Greenland in the west and Novaya Zemlya in the East.

Carex saxatilis subsp. *saxatilis.* This subspecies is widespread in the Scandes, N Scandinavia, Iceland and the Faroes, and local in northern and western Scotland. The species is circumpolar and over most of its area represented by subsp. *laxa.*

Cerastium nigrescens var. *laxum.* This taxon is the non-Arctic component of the previously widely circumscribed *C. arcticum* and was defined by Brysting & Elven (2000). It occurs in the Scandes, Iceland, the Faroes, Scotland and even Mt Snowdon in Wales. The type variety, var. *nigrescens,* is a local endemic on serpentine on the island of Unst in the Shetlands (Brysting & Borgen 2000, Jonsell 2001). Accordingly this species is a North Atlantic endemic, not reaching the Arctic.

Poa ×jemtlandica, the viviparous asexual hybrid between *Poa alpina* and *P. flexuosa*, which contrary to earlier views (Nannfeldt 1937) certainly originated separately in each of its areas, the Scandes, Iceland and central Scotland, where it is very local (Brysting *et al.* 1997, 2000).

Poa flexuosa. Also one of the parental taxa of *P. ×jemtlandica, P. flexuosa*, is a North Atlantic endemic with a distribution very similar to that of *P. × jemtlandica* although wider (south and central Scandes, Iceland and locally in central Scotland; Nannfeldt 1935).

In Scotland the degree of endemism appears to be dramatically lower than in Scandinavia, despite the fact that the regions in some respects are similar. Preston (2003) listed 11 Scottish endemic taxa, 8 of which are taxonomically doubtful, or apomictic microspecies. There are only three with a seemingly undoubted specific status, *Calamagrostis scotica, Cochlearia micacea* and the previously mentioned *Primula scotica,* apparently a parent of the Scandinavian endemic *P. scandinavica.* On the other hand only taxa at some time given species rank are considered by Preston (2003). Even so endemism in Scotland appears low compared with that of Scandinavia.

REFERENCES

ANDERSSON, S. (1990). A phenetic study of *Crepis tectorum* in Fennnoscandia and Estonia. *Nordic J. Botany* **9**: 589–600.

BORGEN, L. (1987). Postglasial evolusjon i Nordens flora – en oppsummering. *Blyttia* **45**: 147–169.

BROCHMANN, C. & ELVEN, R. (1992). Ecological and genetic consequences of polyploidy in arctic *Draba* (Brassicaceae). *Evol. Trends in Plants* **6**: 111–124.

BROCHMANN, C., NILSSON, T. & GABRIELSEN, T. (1996). A classic example of postglacial allopolyploid speciation re-examined using RAPD markers and nucleotide sequences: *Saxifraga osloënsis* (Saxifragaceae). *Symb. bot. upsal.* **31(3)**: 75–89.

BROCHMANN, C., GABRIELSEN, T.M., ELVEN, R. & NORDAL, I. (2003). Glacial survival or tabula rasa. The history of North Atlantic biota revisited. *Taxon* **53**: 417–450.

BRYSTING, A.K. & BORGEN, L. (2000). Isozyme analysis of the *Cerastium alpinum – C. arcticum* complex (Caryophyllaceae) supports a splitting of *C. arcticum* Lange. *Pl. Syst. Evol.* **220**: 199–221.

BRYSTING, A.K. & ELVEN, R. (2000). The *Cerastium alpinum – C. arcticum* complex (Caryophyllaceae): numerical analysis of morphological variation and a taxonomical revision of *C. arcticum* Lange *s. lat. Taxon* **49**: 189–216.

BRYSTING, A.K., ELVEN, R. & NORDAL, I. (1997). The hypothesis of hybrid origin of *Poa jemtlandica* supported by morphometric and isoenzyme data. *Nord. J. Bot.* **17**: 199–214.

BRYSTING, A.K., HOLST-JENSEN, A. & LEITCH, I. (2000). Genomic origin and organization of the hybrid *Poa jemtlandica* (Poaceae) verified by genomic in situ hybridization and chloroplast DNA sequences. *Ann. Bot.* **85**: 439–445.

DAHL, E. (1989). Nunatakk-teorien II. Endemismeproblemet. *Blyttia* **47**: 163–172.

ERICSON, L. & WALLENTINUS, H.G. (1979). Sea-shore vegetation around the Gulf of Bothnia: Guide for the International Society of Vegetation Science, July–August 1977. *Wahlenbergia* **5**: 1–142.

HEDRÉN, M. (1999). Kommentarer om brudkullan och dess ursprung. *Svensk Bot. Tidskr.* **93**: 145–151.

JACOBSSON, A. (2003). Diversity and phylogeography in *Alisma* (Alismataceae), with emphasis on Northern European taxa. Thesis. Univ. of Lund.

JONSELL, B. (1988). Mikroendemism i det baltiska landhöjningsområdet. *Blyttia* **46**: 65–73.

JONSELL, B. (1990a). Evolutionary trends among plants in the Baltic land uplift area. *Sommerfeltia* **11**: 137–146.

JONSELL, B. (1990b). Fjällendemism och annan endemism i Skandinaviens flora. *Blyttia* **48**: 79–81.

JONSELL, B. (1997). Endemism and its backgrounds among vascular plants in Scandinavia. *Sprawd. z pozied. komisji nauk. (Polska Akad. Nauk, Kraków)* **40(2)**: 75–80.

JONSELL, B. ed. (2000). *Flora Nordica 1.* Stockholm.

JONSELL, B. ed. (2001). *Flora Nordica 2.* Stockholm.

JONSELL, B. & KARLSSON, T. (2004). Endemic vascular plants in Norden. In B. JONSELL (ed.) *Flora Nordica. General Volume*: 139–159.

JONSELL, B., NORDAL, I. & ROBERTS, F.J. (2000). *Viola rupestris* and its hybrids in Britain. *Watsonia* **23**: 269–278.

KARLSSON, T. (1986). The evolutionary situation of *Euphrasia* in Sweden. *Symb. Bot. Upsal.* **27(2)**: 61–71.

KNABEN, G. (1954). *Saxifraga osloënsis* n. sp., a tetraploid species of the *Tridactylites* section. *Nytt Mag. Bot.* **3**: 117–138.

KNABEN, G. (1982). Om arts- og rasedannelse i Europa under kvartærtiden 1. Endemiske arter i Nord-Atlanteren. *Blyttia* **40**: 229–235.

LIDÉN, M. (1991). Notes on *Corydalis* sect. *Corydalis* in the Baltic area. *Nord. J. Bot.* **11**: 129–133.

NANNFELDT, J.A. (1935). Taxonomical and plant-geographical studies in the *Poa laxa* group. A contribution to the history of the North European mountain flora. *Symb. Bot. Upsal.* **1(5)**: 1–113.

NANNFELDT, J.A. (1937). On *Poa jemtlandica* (Almqu.) Richt., its distribution and possible origin. A criticism of the theory of hybridization as the cause of vivipary. *Bot. Notiser* 1937: 1–27.

NANNFELDT, J.A. (1940). On the polymorphy of the *Poa arctica* R. Br., with special reference to its Scandinavian forms. *Symb. Bot. Upsal.* **4(3)**.

O'KANE, S.L. JR., SCHAAL, B.A. & AL-SHEHBAZ, I.A. (1996). The origin of *Arabidopsis suecica* (Brassicaceae) as indicated by nuclear rDNA sequences. *Syst. Bot.* **21**: 559–566.

PRESTON, C.D. (2003). Scottish vascular plants in a global context. *Botanical Journal of Scotland* **55**: 7–15.

RICHARDS, J. (1993). *Primula.* Batsford, London.

SOLSTAD, H., ELVEN, R. & NORDAL, I. (1999). Are there too many species and subspecies in the *Papaver radicatum* complex? *Skr. Norske Vidensk.-Akad., Oslo, I. Mat.-Naturvitensk. Kl.* **38**: 281–294.

SOLSTAD, H., ELVEN, R. & NORDAL, I. (2003). Isozyme variation among and within North Atlantic species of *Papaver* sect. *Meconella* (Papaveraceae) and taxonomic implications. *Bot. J. Linn. Soc.* **143**: 225–269.

STEEN, S.W., GIELLY, L.. TABERLET, P. & BROCHMANN, C. (2000). Same parental species, but different taxa: molecular evidence for hybrid origins of the rare endemics *Saxifraga opdalensis* and *S. svalbardensis* (Saxifragaceae). *Bot. J. Linn. Soc.* **132**: 153–164.

TEPPNER, H. & KLEIN, E. (1989). *Gymnigritella runei* spec. nova (Orchidaceae-Orchideae) aus Schweden. *Phyton* **29**: 161–173.

TURESSON, G. (1925). The plant species in relation to habitat and climate. Contributions to the knowledge of genecological units. *Hereditas* **6**: 147–236.

TYLER, T. (2001). Förslag till ny taxonomisk indelning av stångfibblorna (*Pilosella*) i Norden. *Svensk Bot. Tidskr.* **95**: 39–67.

WEBER, H. (1972). Die Gattung *Rubus* im nordwestlichen Europa. *Phanerogamum Monographiae 7.* Cramer, Lehre.

WEIMARCK, G. (1971). Variation and taxonomy of *Hierochloë* (Gramineae) in the northern hemisphere. *Bot. Notiser* **124**: 129–175.

WEIMARCK, G. (1986). *Hierochloë hirta* subsp. *praetermissa* subsp. nova (Poaceae), an Asiatic – E European taxon extending to N and C Europe. *Symb. Bot. Upsal.* **27(2)**: 175–181.

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Evolution in thyme enough? Rapid physiological evolution in response to pollution in two common British plants.

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ABSTRACT

Speciation is often confused with evolution, but taxonomic recognition of a species depends on morphological distinctiveness. We argue that when plants are challenged by rapid and drastic environmental changes such as those resulting from pollution, climate change, or new predators and pathogens, they commonly show dramatic and often sympatric physiological evolution, but because this is normally cryptic ('silent') it is not recognised by taxonomists and so tends to be ignored by conservationists and policy makers. The principle is illustrated by wild thyme *Thymus polytrichus* from metal-rich calaminarian grasslands, and perennial rye-grass *Lolium perenne* from salted road-verges, both of which appear to have evolved tolerance within the last 100 years. Zinc tolerance in *T. polytrichus* is biparentally dominant. We were unable to detect a metabolic cost to this tolerance, or for salt tolerance found in roadside *L. perenne* from several sites. We were unable to induce tolerance by early exposure to salt, and strains that appeared to be innately tolerant were not later affected by this early exposure. In contrast, susceptible strains showed a 'hang-over' effect from early exposure, even when grown in salt-free conditions.

Keywords: *Thymus polytrichus*, *Lolium perenne*, salt tolerance, zinc tolerance, tolerance induction, tolerance costs.

GENERAL INTRODUCTION

While considering the massive contribution that Clive Stace has made to plant taxonomy, not only in the British Isles, AJR fell to contemplating, not for the first time, the very purpose of taxonomy itself. Undeniably, human communication requires living things to be known by reliable, internationally valid names. Unfortunately the multidimensional matrix of morphological variation within which we identify noda for the purpose of this taxonomic recognition does not always fall into neat compartments. It is not surprising that evolution has resulted in patterns of variation which are not readily packaged within a simple system of nomenclature, the philosophical basis of which predates Darwin by more than a century. Consequently, the relationship between a name (taxon) and the limits of the morphological variation which corresponds to this name can be problematical. These problems provide the rationale for the science which was once called 'experimental taxonomy'.

However, in recent years, taxonomy has been called upon to undertake other, quite new tasks whose philosophical bases are very different from the original need to 'give a dog a name'. For instance, detailed studies of DNA sequence variation in well-understood genes are now routinely applied, so that we can trace unambiguously the pathways by which related organisms have evolved. As a consequence, phylogeneticists expect taxonomic frameworks to accurately reflect our understanding of these evolutionary pathways. But, as is discussed in more detail elsewhere in this volume, evolutionary radiation as revealed by the DNA may not always correspond well with either morphological radiation (which may not always be adaptive), or adaptive radiation (including adaptive characters which are not expressed morphologically). Our antiquated taxonomic system is particularly unsuited to a situation where a distinctive derived ('apomorphic') clade which demands generic recognition (for example) is nested within a wider clade for which more than a single generic attribution would be considered inappropriate.

A second 'new' task imposed upon our creaking taxonomic systems has resulted from the sudden rise in prominence of 'biodiversity' in our value judgements, resulting from the extraordinarily influential 'Earth Summit' held at Rio in 1992. The resulting 'Convention on Biological Diversity' has since been ratified by 186 nations, and has led to political systems as diverse as UK 'Biological Action Plans' ('BAPs'), implemented in part at Local Council level (DOE 1994), and international commitments to stabilise loss of Biodiversity made at the World Summit on Sustainable Development in Johannesburg in 2002. Driven by the urgency of the 'mass extinction catastrophe' through which we are currently living, the rate at which we implemented policies designed to conserve biodiversity far outstripped our ability to validate our assessments of that biodiversity, although some attempts are currently being made to redress this (Crane 2003).

Nevertheless, many concerns have been expressed about measures of biodiversity: for instance whether some groups of organisms provide more predictive estimates of biodiversity than others (May *et al.* 1995); and at what taxonomic level (family, genus, species) estimates should be calibrated in various groups (Williams *et al.* 1998). However, one basic tenet, that morphologically-based philosophies of conventional taxonomy should provide the basic unit by which biodiversity is assessed (for instance the species), has rarely been questioned. This dogma involves a number of assumptions, usually tacit, and all of which are essentially untrue. For instance, it is assumed that the species represents a discrete evolutionary end-point; that all species possess roughly the same 'value' as units of evolutionary amplitude; and that species, which are largely based on morphological discontinuities, adequately represent the total significant evolutionary divergence and adaptation that has occurred. It is this last point which forms the subject of the present paper. It is a particular concern that many characters by which species are differentiated in Floras appear to be trivial and non-adaptive, and may have arisen by gene-drift and casual fixation. As a corollary, it seems that many very significant evolutionary characteristics are cryptic or 'silent', in that they are not expressed morphologically and so cannot be described taxonomically (Magurran 1998). In the last 200 years, we have enormously accelerated the rate at which species have become extinct, so that the current era has been compared with mass extinction episodes resulting from environmental catastrophes, such as that which massacred the dinosaurs 65 million years ago (Myers & Knoll 2001). What is much less often stated is that by creating new environments, and by bringing together in open habitats species which evolved in isolation, we have simultaneously greatly increased the rate at which new biological entities have evolved. We have no 'base-line' from earlier times against which

hypothetical changes in rates of evolutionary diversification after the industrial and agricultural eras can be assessed, so that such assertions of recent increases in the rate of evolutionary change are essentially untested, but this does not make them untrue.

Nevertheless, there are numerous 'headline' examples where morphologically distinctive new species have evolved recently, usually as a result of long-distance migration, hybridity and polyploidisation, *Spartina anglica* C.E.Hubb. and *Senecio cambrensis* Rosser in the UK alone for instance. An even greater volume of rapid contemporary evolution may have passed largely unnoticed by students of biodiversity, because it does not in the main involve morphological changes (is 'silent') and so has not been recognised taxonomically.

A vital function of measures of biodiversity is predictiveness, so that we can construct models which show how potentially ameliorative shifts in environmental policy might alter forecast rates of extinction (Pimm & Askins 1995, Pitman *et al.* 2002). Such models usually assume zero evolutionary change, so that the possible role played by 'Red Queen' in allowing threatened organisms to escape recent environmental damage by adaptation to new niches is generally ignored. Yet the literature of ecological genetics contains many thousands of examples where such evolution has occurred, unrecognised by taxonomy or measures of biodiversity. This paper reports two previously unreported but rather typical examples of such recent physiological 'silent' evolution of plants in response to polluting activities by man.

ZINC TOLERANCE IN THYME

INTRODUCTION

Historic mining has produced widespread lead, cadmium and zinc contamination of the fluvial deposits of the South Tyne and Tyne river basins in north-east England (Hudson-Edwards *et al.* 1996). Although some mining for lead has occurred over 2000 years, mining for zinc started in about 1880 and peaked between 1900 and 1910, virtually ceasing by 1920 (Macklin & Smith 1990). Most contaminated deposits now lie above normal flood level, but remain phytotoxic so that plant communities are very open and carry a specialised metallophyte flora. This habitat, from which more than 40 species have been recorded in this region, seems to be equivalent to OV37 '*Festuca ovina–Minuartia verna* community' in the National Vegetation Survey (Rodwell 2000), although the latter states that only 'a few' such river gravel sites are known (in fact more than 50 have been recorded) and admits that no such sites had been included in their survey. Locally, such sites have become known as 'calaminarian grasslands'.

We assume that most of the metallophyte species that occur here, including *Thlaspi caerulescens* J.S. & C. Presl, *Minuartia verna* (L.) Hiern., *Viola lutea* Huds., *Silene vulgaris* Garcke and *Armeria maritima* (Miller) Willd. (Richards *et al.* 1989), show unusually high levels of tolerance to metals such as lead and zinc. In the present context it is of interest to discover whether such tolerance is constitutive (typical of the species as a whole, as may be the case for the species above); inducible; or has evolved recently from relatively susceptible races on non-toxic habitats (Baker 1987). In order to demonstrate metal tolerance, we would expect that after transplantation to non-polluted soil, propagants of individuals originating from metal-rich habitats would grow more readily in conditions with high levels of the metal

under investigation than did individuals from low metal (control) habitats, and that this tendency could be inherited between generations.

We suspect that metal tolerance may have evolved within a number of species on Tyne calaminarian grasslands within the last 100 years, although it is possible that these habitats have been colonised by metal-tolerant strains of longer standing. In no case has a morphologically distinctive ecotype arisen. In this case, our investigations centred on zinc tolerance in wild thyme *Thymus polytrichus* A. Kerner ex Borbas subsp. *britannicus* (Ronn.) Kerguélen which is a characteristic component of the most apparently toxic parts of this habitat, but elsewhere is typical of non-polluted soils.

ORIGIN OF MATERIAL AND MEASUREMENT OF ZINC TOLERANCE

To test for the occurrence of zinc tolerance in thyme, propagants of adult plants from the most apparently toxic parts of four calaminarian grassland sites (M1–M4) and two control sites on limestone grassland (C1, C2) (Table 1) were brought into cultivation and established under glass where they were kept in active growth throughout the year. Total and extractable average soil concentrations of Zn, Pb and Cd, average soil pH, and extractable P are also given for each site, for soils taken from amongst the roots of the plants propagated (methods in Whitfield 2002). To measure tolerance to a toxic metal such as zinc, clones were propagated from semi-hardened cuttings, and root growth of these ramets studied when suspended in a liquid medium (solution of 0.5 g l^{-1} calcium nitrate (0.72 g l^{-1} $Ca(NO_3)_2.4H_2O$ in distilled water)) in transparent containers (root extension technique, Wilkins 1957). Growth rate in this medium was studied for 2-3 weeks and when the rate became constant was recorded between days 3 and 6 in the medium (a) and between days 9 and 12 in the medium with 0.1 mg 1^{-1} Zn^{2+} (as $ZnS0_4.7H_20$) added on day 6 (b). Tolerance index (TI) for a particular clone was the average of the b/a ratios. Absolute growth rates for days 9–12 (TI_{ab}) were also compared to avoid the possibility of scalar bias. To study inheritance of tolerance, controlled crosses were made onto pollen sterile (female) individuals in this gynodioecious species, using parents of known TI, and the TI of their offspring investigated using the same technique.

RESULTS

The average tolerance index (TI) for clones did not differ significantly between the toxic sites, but in every case was significantly greater than for the control sites, indicating that all the clones tested in all the toxic sites were tolerant to zinc (Table 2). For plants from toxic and control sites, there was a loose but significant relationship between the TI displayed by the individual and the extractable concentration of zinc taken from amongst the roots of that plant ($r^2 = 0.15$, $F_{1,58} = 9.82$, $P < 0.005$), suggesting that some selection for individuals of a given level of tolerance occurs between microsites within the toxic areas.

Offspring do not differ significantly in TI from the more tolerant parent, whether the male or female parent to the cross (Fig. 1), but when they result from crosses between a more tolerant parent and a less tolerant parent, their tolerance is significantly higher than that of the latter. Consequently, a pattern of dominance for zinc tolerance is clearly demonstrated, although offspring tend to have a higher TI than the average of their parents TI.

Root growth rate in calcium nitrate (reading (a)) in individual clones shows no relationship to TI (Fig. 2), so that there is no indication from this technique that zinc tolerance causes the plant a metabolic cost.

TABLE 1. LOCATIONS FROM WHICH EXPERIMENTAL PLANTS OF *THYMUS POLYTRICHUS* WERE TAKEN, WITH AVERAGE LEVELS OF TOTAL AND EXTRACTABLE METALS, PHOSPHORUS (P), CARBON (C) AND PH OF SOILS TAKEN FROM THEIR ROOTS. SITE AND SOIL CHARACTERISTICS (MEAN±SE).

	Control sites*		**Heavy-metal-contaminated sites**			
	C1	**C2**	**M1**	**M2†**	**M3**	**M4**
Site name/ grid reference	Gunnerton NY 915 750	Sherburn Hill NZ 333 418	Warden NY 900 665	Bardon Mill NY 780 643	Broomhouse NY 695 628	Fourstones NY 893 672
No. of samples	5	10	23	9	23	16
Total metals (mg kg^{-1})						
Cd	1.88±0.16 a	nd	2.68±0.10 b	5.20±0.33 c	5.08±0.40 c	9.43±0.34 d
Pb	14.42±8.97 a	nd	573.8±11.02 b	821.4±35.76 c	966.7±32.05 d	1358±29.37 e
Zn	127.3±23.6 a	nd	912.8±43.52 b	1753±86.69 c	2024±145.8 c	3641±84.90 d
Extractable metals (mg kg^{-1})						
Cd	0.72±0.17 a	0.12±0.02 b	1.55±0.08 c (n=15)	3.66±0.36 d	2.50±0.15 c (n=15)	3.84±0.13 d
Pb	8.37±1.42 a	10.80±1.10 a	299.9±7.61 b	540.2±39.82 c	525.4±15.76 c	513.7±16.15 c
Zn	26.51±8.86 a	5.45±0.62 a	286.4±10.06 b	584.3±51.15 c	616.5±29.35 c	937.3±30.38 d
Solution Zn (mg l^{-1})	nd	nd	0.32±0.08 a (n=6)	nd	0.26±0.04 a (n=7)	1.06±0.21 b (n=2)
Extractable P (mg kg^{-1})	12.84±2.96 a,d	4.95±0.71 a	2.95±0.26 b	8.53±1.63 c,d	2.86±0.22 b	3.17±0.20 b
Total C (g kg^{-1})	59.87±7.61 a,f	102.2±3.33 b	24.64±4.85 c	42.48±6.36 d,f	22.66±1.48 c	22.39±1.65
pH (mean, range)	7.1 (7.0–7.1)	7.6 (7.4–7.7)	6.6 (6.2–7.0)	6.3 (6.0–6.7)	6.9 (6.5–7.2)	6.8 (6.4–7.0)

nd=not determined. Within each row, values followed by the same letter are not significantly different (P<0.05, t-test).

* Gunnerton Crag: short grassland with thyme growing mainly in thin soil overlying rocky outcrops.
Sherburn Hill: sparsely vegetated hilltop with diserete patches of thyme growing on thin, stony, limestone soils.

† Mean values for soils sampled at a depth of 20–30 cm (total/extractable): Cd 7.26/2.31, Pb 1417/540.4, Zn 2523/406.6 mg kg^{-1}.

TABLE 2. AVERAGES OF ZINC TOLERANCE INDEX (TI%) AND TI_{AB} (ABSOLUTE GROWTH RATE IN ZINC SOLUTION) FOR *THYMUS POLYTRICHUS* FROM CONTROL (C1, C2) AND METALLIFEROUS (M1-M4) LOCATIONS TOGETHER WITH SIGNIFICANCE OF DIFFERENCES AGAINST C1 AND C2 RESPECTIVELY.

Site	**No. of clones**	**TI (%)**	***P* vs C1, C2 (*t*-test)**	**TI_{ab} (mm)**	***P* vs C1, C2 (ANOVA*)**
C1	3	6.67±1.20 a		0.55±0.14 a	
C2	10	10.44±2.92 a		1.49±0.34 a	
M1	14	22.02±3.69 b	<0.01, <0.05	4.26±0.82 b	<0.001, <0.01
M2	8	24.95±4.16 b	<0.05, <0.01	3.24±0.61 b	<0.001, <0.01
M3	17	29.50±4.07 b	<0.001, <0.001	4.11±0.55 b	<0.0001, <0.001
M4	11	25.84±2.20 b	<0.01, <0.001	3.22±0.39 b	<0.001, <0.01

Within columns, values followed by the same letter are not significantly different ($P<0.05$).
*Values log-transformed for ANOVA: $F_{5,57}=6.17$, $P<0.0001$.

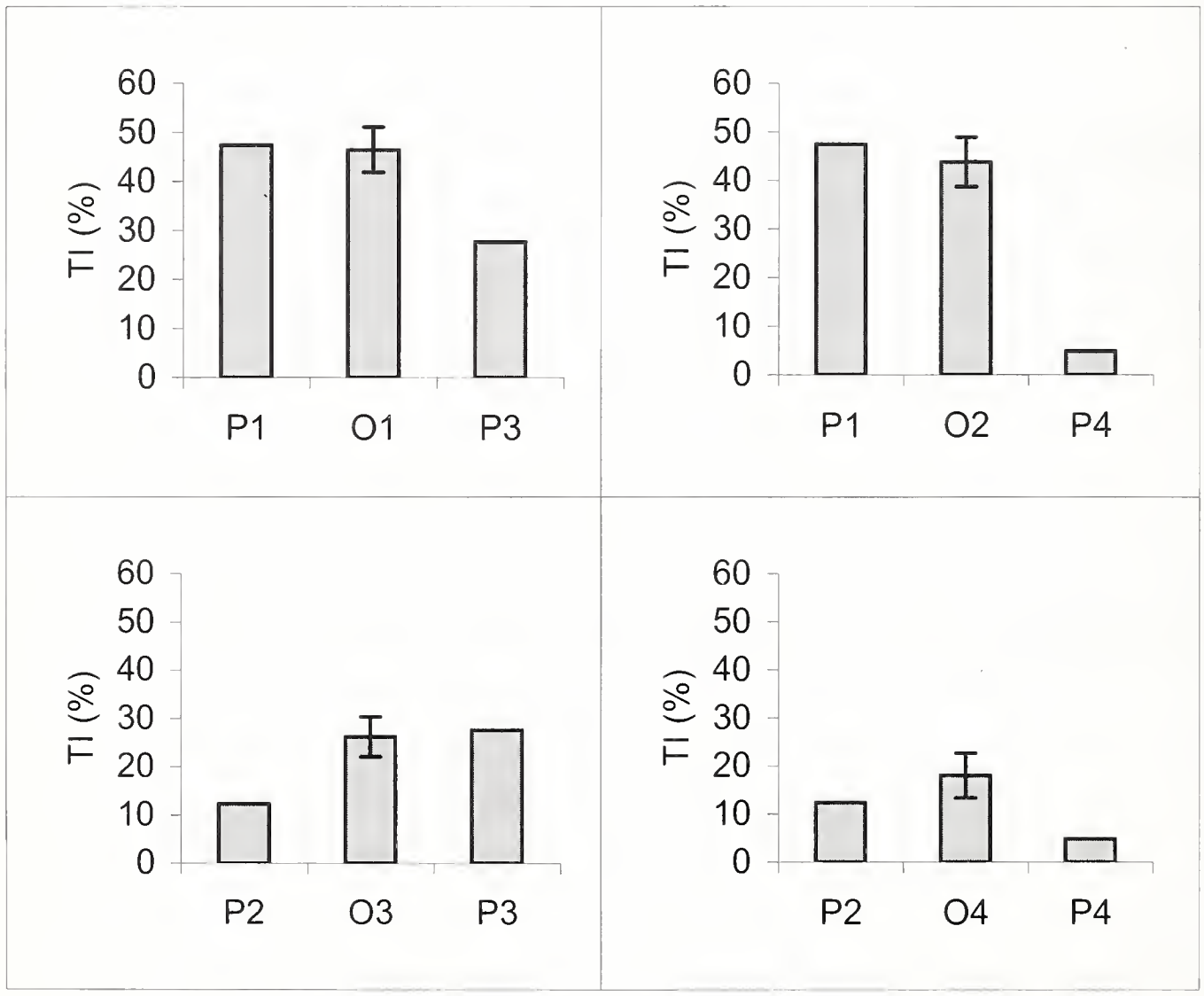


FIGURE 1. Results of four crosses between *Thymus polytrichus* individuals with a high (P1), medium TI (P3) and low TI (P2, P4) average zinc tolerance index (TI%). Male parent to cross on left. Average TI of offspring (O1-O4) shown with standard error. Inheritance of TI appears to be fully dominant and reciprocal (* $P < 0.05$, ** $P < 0.005$).

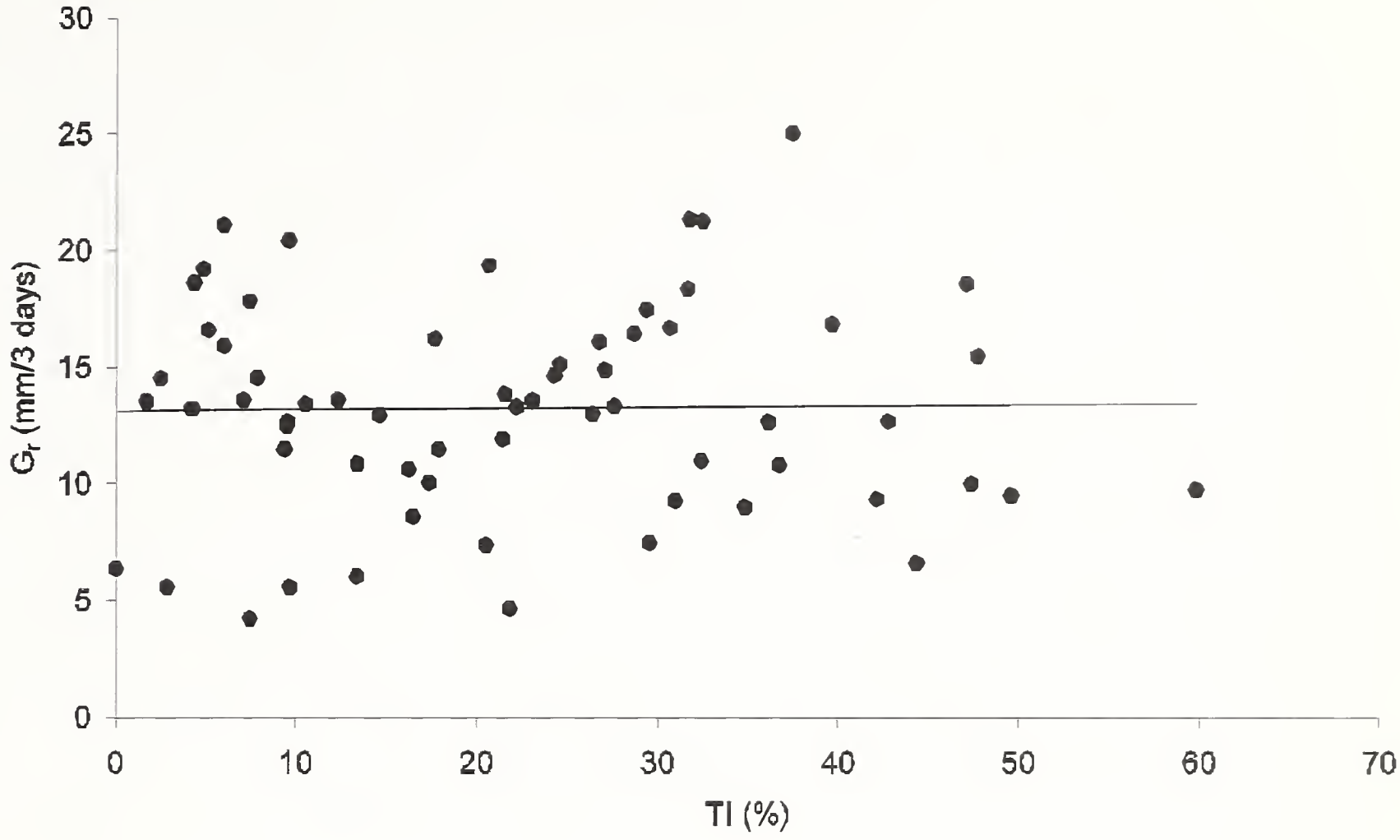


FIGURE 2. Average zinc tolerance index (TI%) (x axis) of each clone of *Thymus polytrichus* and root growth rate of each clone in calcium nitrate solution in the absence of zinc (y axis) are not correlated ($r^2 = 0.003$, $F_{1,62} = 0.017$), suggesting that there is no innate cost to tolerance.

SALT TOLERANCE IN RYE-GRASS

INTRODUCTION

De-icing salt (mostly sodium chloride) has been used on British roads since the 1940s, although heavy and widespread use with mechanical application did not occur until the 1960s (HMSO 1968). Deposition levels of 34g/m^2 per individual application have been suggested (Davison 1971). De-icing salt is spread into the roadside environment by run-off, infiltration, use of snow-ploughs, and splash/spray from vehicles. However, salt levels decrease exponentially with distance from road and more than 90% of salt deposition occurs within 20 m of the road, so that in Northumberland, UK, Scott (1985) found the highest soil conductivities at 0.5 m from the verge; at 1.5 m conductivities were scarcely raised beyond those typical away from roads. Scott also recorded a striking annual fluctuation of soil conductivity in roadside soils, the greatest inferred salinities occurring in February.

High salinity affects plants in many ways, by decreasing osmotic potential within the plant, through the toxicity of sodium and chlorine ions (Westing 1969, Norrstrom & Bergstedt 2001) and by altering soil pH, nutrient availability, and structure (Davison 1971). By replacing other cations, sodium causes soil particles to lose their capability for aggregation and flocculation, so that the ability of the soil to hold water and oxygen decreases. Consequently, salt can be both the primary and secondary source of plant decline (Dobson 1991).

As a result, it is frequently found that 'grassland' immediately adjacent to the road is essentially devoid of vegetation, particularly where trunk roads on which vehicle speeds are relatively high are not provided with a protective 'hard shoulder' lane. In these bare areas it is possible to find isolated individuals of several species of grass. *Puccinellia distans* (Jacq.)Parl. naturally occurs on salt-marshes and is well-known to have colonised salted verges throughout much of Britain (Scott & Davison 1982) so it is expected to be constitutively salt-tolerant. Most other individuals occurring in these circumstances are either *Poa pratensis* L. or *Lolium perenne* L. As reproduction in *Poa pratensis* is primarily apomictic, we decided to concentrate on perennial rye-grass *Lolium perenne*.

Lolium perenne is a variable outcrossing tetraploid. When many thousands of seedlings were screened, a very few proved to be salt tolerant as assessed by their capability for root growth in a saline solution (Ashraf *et al.* 1986a) and this capability was associated with greater dry matter productivity in sand culture with added saline when compared with low tolerance individuals (Ashraf *et al.* 1986b). Further selection of 'salt-tolerant' lines led to individuals which performed even better in saline conditions (Ashraf 1990). Nevertheless, although it has been shown that *Lolium perenne* can have the potential to vary genetically for tolerance to salt, there has been no indication that this has actually led to the evolution of wild strains which have been able to colonise salt-polluted soils.

ORIGIN OF MATERIAL AND MEASUREMENT OF SALT TOLERANCE

To test the hypothesis that the isolated individuals of *Lolium perenne* which occur in salt-polluted bare patches were salt tolerant, we took four such individuals ('verge') into cultivation and tested them in comparison with four individuals occurring about 15 m from the carriageway in a grass sward of healthy appearance ('control'). In April 2002, samples were dug up from the verges of the A167, a dual carriageway in the suburbs of Newcastle upon Tyne at NZ215.672. Soil was sampled from amongst the roots of each clone and the salinity estimated by conductivity using the technique of Davison (1971) which is adapted from that recommended by the Soil Survey of England and Wales.

Plants were then washed clean with tap water and each divided into 40 single-shoot tillers each with a fragment of root. These tillers were individually grown under glass in standard volume plastic pots in a single carefully mixed batch of compost approximating to John Innes 2 watered from below for two weeks; those few tillers which failed to establish and grow were abandoned.

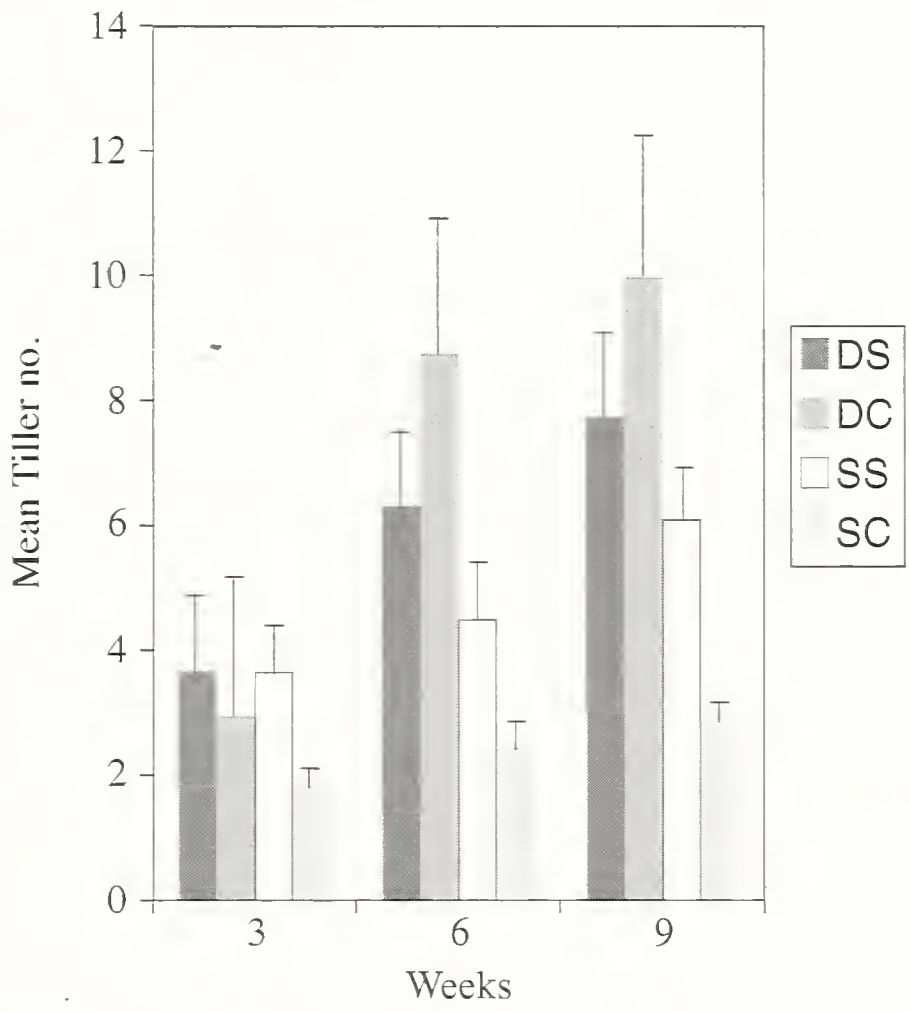
The experimental period lasted nine weeks, during which time batches of 80 tillers were each evenly watered from above in a fine spray every three days using 10 l of either distilled water or 0.15M sodium chloride (8.76g/l) ('saline'). Soil conductivities were checked at three week intervals, and on average increased from 8 to17 mS in pots watered with saline for nine weeks, but remained below 1 mS at weeks three and six in pots watered with distilled water. In a second experiment, plants which had been treated with saline for the first three weeks only, and the first six weeks only, and were watered with distilled water for the remainder of the nine weeks were compared with those watered only with distilled water. Also, plants watered with saline for only the final three weeks, and the final six weeks were compared with those which had been treated with saline for all nine weeks.

At harvest, the performance of each initial tiller was judged in two ways: final tiller number; and above-ground dry weight.

RESULTS

The average conductivities of soils from amongst roots of plants collected in mid April varied from 0.9 to 2.1 mS for verge samples (mean 1.28 mS), but from only 0.3 to 0.5 mS in control samples (mean 0.39 mS).

At harvest, verge plants watered with saline had grown on average twice as many tillers as control plants treated with saline, and the number of tillers formed by verge plants in saline did not differ significantly from those watered with distilled water. After treatment with distilled water we found no significant difference between the number of tillers formed by verge and control plants, so that this result revealed no appreciable metabolic cost to verge plants compared to control plants in the absence of saline (Fig. 3). Analysis of dry weight by GLM showed a significant interaction between source (verge or control) and treatment (saline or distilled water) ($F_{1,273}$= 20.53, $P < 0.001$), thereby demonstrating that verge plants showed more tolerance to saline than control plants. There was no significant difference in tolerance noted between clones from the same source (Table 3).



DS = Salt exposed plant grown in distilled water

DC = Control plants grown in distilled water

SS = Salt exposed plants grown in saline solution

SC = Control plants grown in saline solution

FIGURE 3. Mean and standard error of tiller number developed from single tillers of *Lolium perenne* over nine weeks, indicating verge plants (DS, SS) and control plants (DC, SC) grown in saline (SS, SC) and distilled water (DS, DC). Verge plants grow much better than control plants in saline and do not differ from those grown in distilled water.

TABLE 3. ANOVA, EFFECT OF SOURCE AND TREATMENT ON DRY WEIGHT IN *LOLIUM PERENNE*.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	1	6.82	6.27	6.27	80.63	<0.001
Source	1	2.83	2.78	2.78	35.79	<0.001
Clone	6	0.32	0.25	0.04	0.55	0.772
Interaction, treatment and source	1	0.50	0.59	0.59	7.57	0.007
Interaction, treatment and clone	6	0.74	0.74	0.12	1.59	0.158
Error	103	8.00	8.00	0.08		
Total	118	19.2				

TABLE 4. ANOVA, EFFECT OF SOURCE AND TIME OF TREATMENT ON DRY WEIGHT IN *LOLIUM PERENNE* WATERED WITH SALINE.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Source	1	1.48	1.37	1.37	18.9	<0.001
Clone	6	0.87	0.80	0.13	1.85	0.097
Time	2	1.40	1.43	0.71	9.87	<0.001
Interaction, time and source	2	0.02	0.03	0.01	0.18	0.832
Interaction, time and clone	12	0.38	0.38	0.32	0.44	0.942
Error	112	8.10	0.07	0.07		
Total	135					

TABLE 5. ANOVA, EFFECT OF SOURCE AND TIME OF PRETREATMENT WITH SALINE ON DRY WEIGHT IN *LOLIUM PERENNE* WATERED WITH DISTILLED WATER.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Source	1	8.62	8.78	8.78	143.2	<0.001
Clone	6	1.76	1.67	0.28	4.54	<0.001
Time	2	2.43	2.41	1.21	19.7	<0.001
Interaction, time and source	2	0.12	0.13	0.06	1.06	0.349
Interaction, time and clone	12	0.66	0.66	0.05	0.89	0.555
Error	113	6.93	6.93	0.06		
Total	136	20.5				

Control plants which had been pretreated with saline for the first three and six weeks of the experiment performed less well over the final three weeks of the experiment in saline than plants which had been pretreated with distilled water and then tested with saline (Table 4). In contrast, verge plants were not affected by this early exposure to saline. We concluded that tolerance to salt is not induced by exposure to salt in this case, but that salt tolerance expressed by verge plants is probably under genetic control.

When control plants are subjected to saline in early weeks, their performance in the final three weeks of the experiment in distilled water is affected compared with those watered with distilled throughout, but verge plants show no such 'hang-over' effect from this early exposure to saline (Table 5).

DISCUSSION

We have shown for the first time that wild thyme *Thymus polytrichus* growing on metal-polluted river gravels are tolerant to zinc, and that perennial rye-grass *Lolium perenne* on heavily salted road-verges is salt tolerant. The pollution of both these habitats has resulted directly or indirectly from the activities of man. We believe that the following protocols must be satisfied if the occurrence of adaptive tolerance is to be established:

- in the concentrations in which it typically occurs in polluted habitats, the pollutant should be sufficiently phytotoxic that vegetational cover is open and bare;
- concentrations of the pollutant should normally and consistently be at a much higher level in polluted habitats than in those where susceptible 'wild type' plants (which are used as controls and which are deemed to be typical of stock from which tolerant forms evolved) are found;
- experimental plants should be grown in pollutant-free conditions prior to the experimentation, to lessen the possibility of 'hang-over' or inducible' effects from prior exposure to the pollutant;
- replicated propagants taken from polluted habitats should for every clone grow significantly better in the presence of the polluting substance in otherwise uniform controlled conditions than those taken from most or all clones from an unpolluted ('control') source(s);
- in the absence of the polluting substance (control conditions), propagants from polluted habitats would not however be expected to grow better than those from control habitats;
- EITHER the heritability of the tolerance should be established by comparing the tolerance of the offspring of two susceptible parents with those of two tolerant parents, OR the potential for tolerance induction is investigated by comparing the tolerance of clones of control origin which have, and which have not, been pretreated with the pollutant.

We believe that these conditions have been met in each of the two cases we have investigated. These two examples differ in many attributes. One species is a woody dicotyledon, the other a herbaceous monocotyledon. In one case it is a metal that is the toxin, in the other the toxicity is more indirect in the sense that soil and soil water properties are affected by salt in complex ways. Metal pollution on river gravels is decreasing, but this is probably untrue of roadside salt deposition. Nevertheless, we are more struck by a number of features shared by both cases.

- The polluting activities are relatively recent, in that most zinc pollution of the River South Tyne has occurred in the last 100 years, and most roadside salting in the last 40 years.
- Thus, we suppose that in both cases, the occurrence of tolerant individuals in these habitats is also very recent. However, we need to explore the likelihood that these species could have been exposed to the pollutant at an earlier date, and that tolerant plants could have dispersed from there to the present sites. In the case of *Thymus polytrichus*, 'natural' metalliferous habitats occur in the River South Tyne catchment, but are exceptionally localised (Hudson-Evans *et al.* 1996). *Lolium perenne* is not usually a constituent of naturally saline conditions (but T. Rich (*in litt.)* provides one anecdotal example of *L. perenne* in a salt-marsh from, appropriately enough, Rye in Sussex).
- Tolerant genotypes have spread into other suitable localities. Zinc tolerant individuals of *Thymus polytrichus* are known to occur at a number of sites, possibly as the result of colonisation of polluted habitats by water-borne seed from an upstream founder where evolution of tolerance originated. Although salt tolerance of *Lolium perenne* has only been tested at one site, plants occur within similar heavily polluted areas throughout greater Newcastle upon Tyne and we expect these also to be salt tolerant and to have been spread between sites by the movement of vehicles.
- We can establish no apparent cost expressed as vigour associated with the tolerance.
- Evolution has been for physiological features, and as far as we are aware there are no morphological features that differentiate between tolerant and susceptible individuals in either case.

It is likely that such examples of very recent 'silent' physiological evolution are very common. Although many already exist in the literature, many more probably lie undetected in the absence of experimental work. Physiological evolution may not only occur in response to chemical pollution, but also to climate change, or to the introduction or evolution of 'new' predators, pathogens and competitors, and such evolution is likely to be much more significant in future considerations concerning the survival of biodiversity, than are the few 'headline' examples of new distinctive hybridogenous species such as *Senecio cambrensis.* We need to consider whether 'alpha taxonomy' alone will be adequate to answer the needs of future conservationists. By attempting to quantify 'silent' physiological evolution more accurately, perhaps the time has come to challenge the popular conception that biodiversity and taxonomic diversity are synonymous.

REFERENCES

ASHRAF, M. (1990). Selection for salt tolerance and its genetic basis in perennial rye-grass (*Lolium perenne* L.). *Hereditas* **113**: 81–85.

ASHRAF, M., MCNEILLY, T. & BRADSHAW, A.D. (1986a). Tolerance of sodium chloride and its genetic basis in natural populations of four grass species. *New Phytologist* **103**: 725–734.

ASHRAF, M., MCNEILLY, T. & BRADSHAW, A.D. (1986b). The response of selected salt-tolerant and normal lines of four grass species to NaCl in sand culture. *New Phytologist* **104**: 453–461.

BAKER, A.J.M. (1987). Metal tolerance. *New Phytologist* **106** (suppl.) 93–111.

CRANE, P. (2003). *Measuring Biodiversity for Conservation.* Royal Society of London, Policy Document 11/03.

DAVISON, A.W. (1971). The effect of de-icing salt on roadside verges. I. Soils and plant analysis. *Journal of Applied Ecology* **8**: 555–561.

DOBSON, M.C. (1991). De-icing salt damage to trees and shrubs. *Forestry Commission Bulletin* **101**. HMSO, London.

DOE (Department of the Environment) (1994). *Biodiversity: the UK Action Plan.* HMSO, London, UK.

HMSO (1968). *Salt treatment of snow and ice on roads*. Road Note 18, edn 2. Road Research Laboratory, Crowthorne, Berkshire, UK.

HUDSON-EVANS, K.A, MACKLIN, M.G., CURTIS, C.D. & VAUGHAN, D.J. (1996). Processes of formation and distribution of Pb-, Zn-, Cd-, and Cu-bearing minerals in the Tyne basin, north-east England: implications for metal-contaminated river systems. *Environmental Science and Technology* **30**: 72–80.

MACKLIN, M. & SMITH, R.S. (1990). Historic riparian vegetation development and alluvial metallophyte plant communities in the Tyne basin, north-east England. In: J.B. Thornes, (ed.), *Vegetation and erosion: processes and environment.* pp. 239–256. John Wiley & Sons, Chichester.

MACURRAN, A.E. (1998). Population differentiation without speciation. *Philosophical Transactions of the Royal Society of London* B **353**: 275–286.

MAY, R.M., LAWTON, J.H. & STORK, N.E. (1995). Assessing extinction rates. In: J.H. Lawton & R.M. May, (eds), *Extinction Rates,* pp. 1–24. Oxford University Press, Oxford.

MYERS, N. & KNOLL, A.H. (2001). The biotic crisis and the future of evolution. *Proceedings of the Natural Academy of Sciences*, Washington DC **98**: 5389–5392.

NORRSTROM, A.C. & BERGSTEDT, E. (2001). The impact of road de-icing salt (NaCl) on colloid dispersion and base cation pools in roadside soils. *Water, Air and Soil Pollution* **127**: 281–299.

PIMM, S.L. & ASKINS, R.A. (1995). Forest losses predict bird extinctions in eastern North America. *Proceedings of the Natural Academy of Sciences*, Washington DC **92**: 9343–9347.

PITMAN, N.C.A, JORGENSEN, P.M., WILLIAMS, R.S.R., LEON-YANEZ, S. & VALENCIA, R. (2002). Extinction-rate estimates for a modern neotropical flora. *Conservation Biology* **16**: 1427–1431.

RICHARDS, A.J., LEFEBVRE, C., MACKLIN, M. NICHOLSON, A. & VEKEMANS, X. (1989). The population genetics of *Armeria maritima* (Mill.) Willd. on the River South Tyne. *New Phytologist*. **112**: 281–293.

RODWELL, J.S. (2000). *British Plant Communities* Vol. 5. Cambridge University Press, Cambridge, UK.

SCOTT, N.E. (1985). *The updated distribution of maritime species on British roadsides.* Ph.D. thesis, University of Newcastle upon Tyne.

SCOTT, N.E. & DAVISON, A.W. (1982). De-icing salt and the invasion of road verges by maritime plants. *Watsonia* **14**: 41–52.

WESTING, A.H. (1969). Plants and salt in the roadside environment. *Phytopathology* **59**: 1174–1180.

WHITFIELD, L. (2002). *Heavy metal tolerance and mycorrhizal colonisation in* Thymus polytrichus *A. Kerner ex Borbas ssp.* britannicus *(Ronn.) Kerguelen (Lamiaceae).* Ph.D. thesis, University of Newcastle upon Tyne.

WILKINS, D.A. (1957). A technique for the measurement of lead tolerance in plants. *Nature* **180**: 37–38.

WILLIAMS, P.H., GASTON, K.J. & HUMPHRIES, C.J. (1998). Mapping biodiversity value world-wide: combining higher-taxon richness from different groups. *Proceedings of the Royal Society of London* B **264**: 141–148.

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Some thoughts about the future of electronic floras

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In Europe a few countries already have electronic floras: Swiss (Lauber & Wagner 1997), Sweden (Anderberg 1998), the Netherlands (Van der Meijden 1999), Germany (Seybold 2001), Bulgaria (Petrova 2002), British Isles (Stace 2004).

The advantages of electronic floras are that they contain more illustrations than any printed book may do (for the British one: 7000 colour slides and 2000 line drawings), as well as distribution maps. In future editions illustrations can be added or replaced easily, and maps can be updated easily as well. An extra feature of the British and the Dutch flora is the fact that they contain all basal distribution records, which enables the user to withdraw those data for a certain area; one can easily get a list of all species known in a certain square or selection of squares.

A disadvantage is that a computer is (still) heavier than a book. I expect that printed floras will remain to be used in the future, especially in the field, and that electronic floras will be used at home or in the base camp.

Nearly all electronic floras use a traditional dichotomous key, usually literally the same as in the printed book. *The Interactive Flora of the British Isles* (Stace 2004) has made the standard dichotomous keys much more user-friendly by a series of devices. Firstly, the user is confronted with only the relevant couplet, and once the choice is made he is taken straight to the next relevant couplet (Fig. 1). Secondly, at any point, the user is told how many taxa he has already eliminated and how many taxa remain as possibly correct (Fig. 1, 3); the latter can be listed and by selecting any of them a picture of that taxon can be displayed (Fig. 5). Thirdly, at any point the user can obtain a list of the decisions that he has already made, and after studying them he can back-track to one that he thinks might have been made in error, and change it accordingly to take him on a different pathway (Fig. 3). Finally, by clicking on any term unknown to the user he is taken straight to its definition in the Glossary (Fig. 1, 2); this facility applies equally to the plant descriptions as well. By these means it is hoped that the two major problems encountered by beginners, the complex terminology and the mysteries of dichotomous keys, can be alleviated.

Next to this, the electronic floras sometimes include multi-access keys, which are more easily used on a computer than in a book. At present, most multi-access keys are available for difficult groups only. The challenge, however, is to make really user-friendly general keys, like those that Richard Pankhurst is making for the British flora.

Another solution may be the construction of a kind of sorting program, like the one in the Dutch flora. The characters used there are simple: leaves (compound, simple; length-width ratio, arrangement), flower colour and actual date of finding the plant in flower and its height, habit and habitat. With the help of these easy characters the user gets a quick selection of species; he or she can click on their names, attempting to find the proper species.

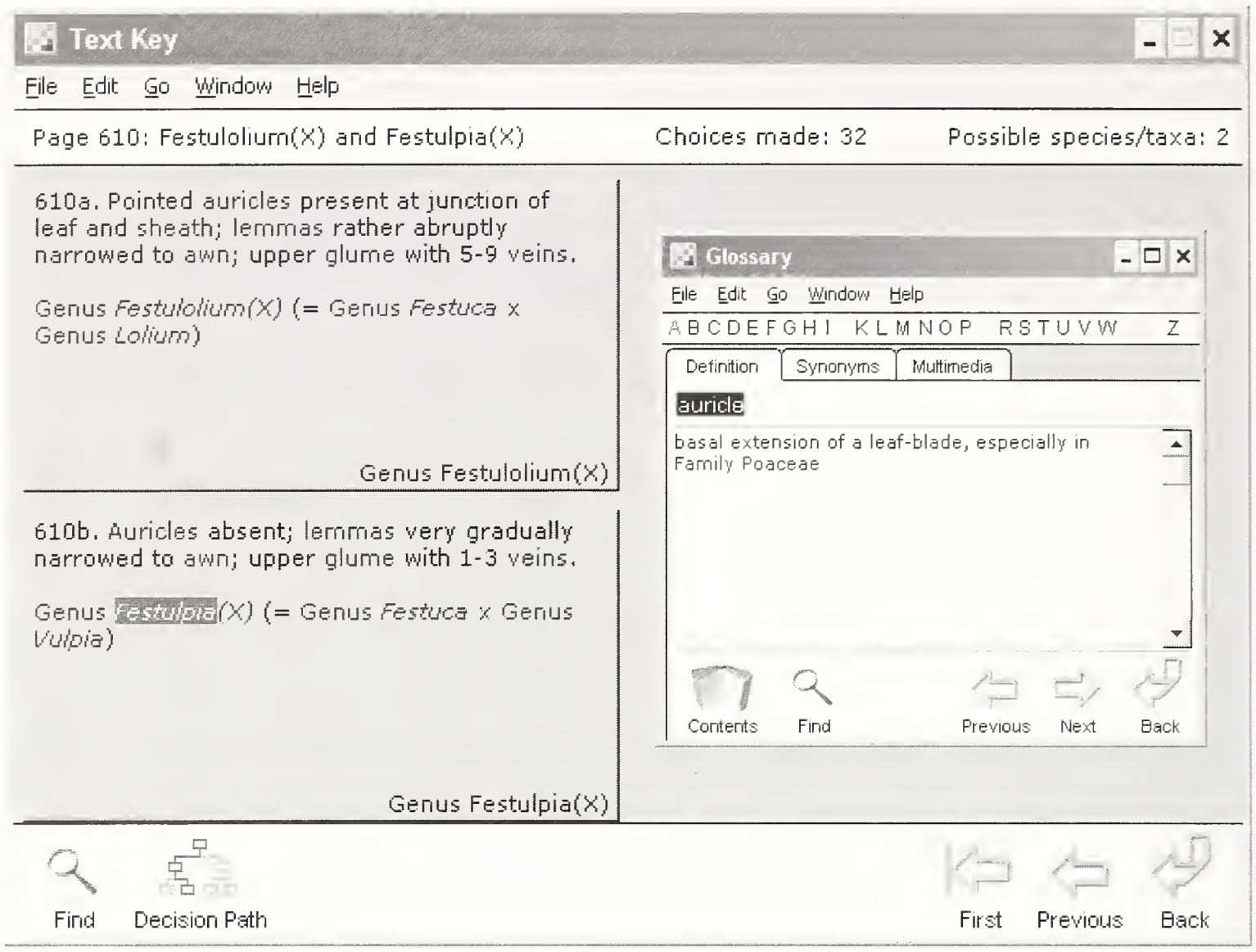

Figure 1. Page from Stace (2004) showing a text key couplet (with glossary screen inset), decisions made and number of taxa remaining.

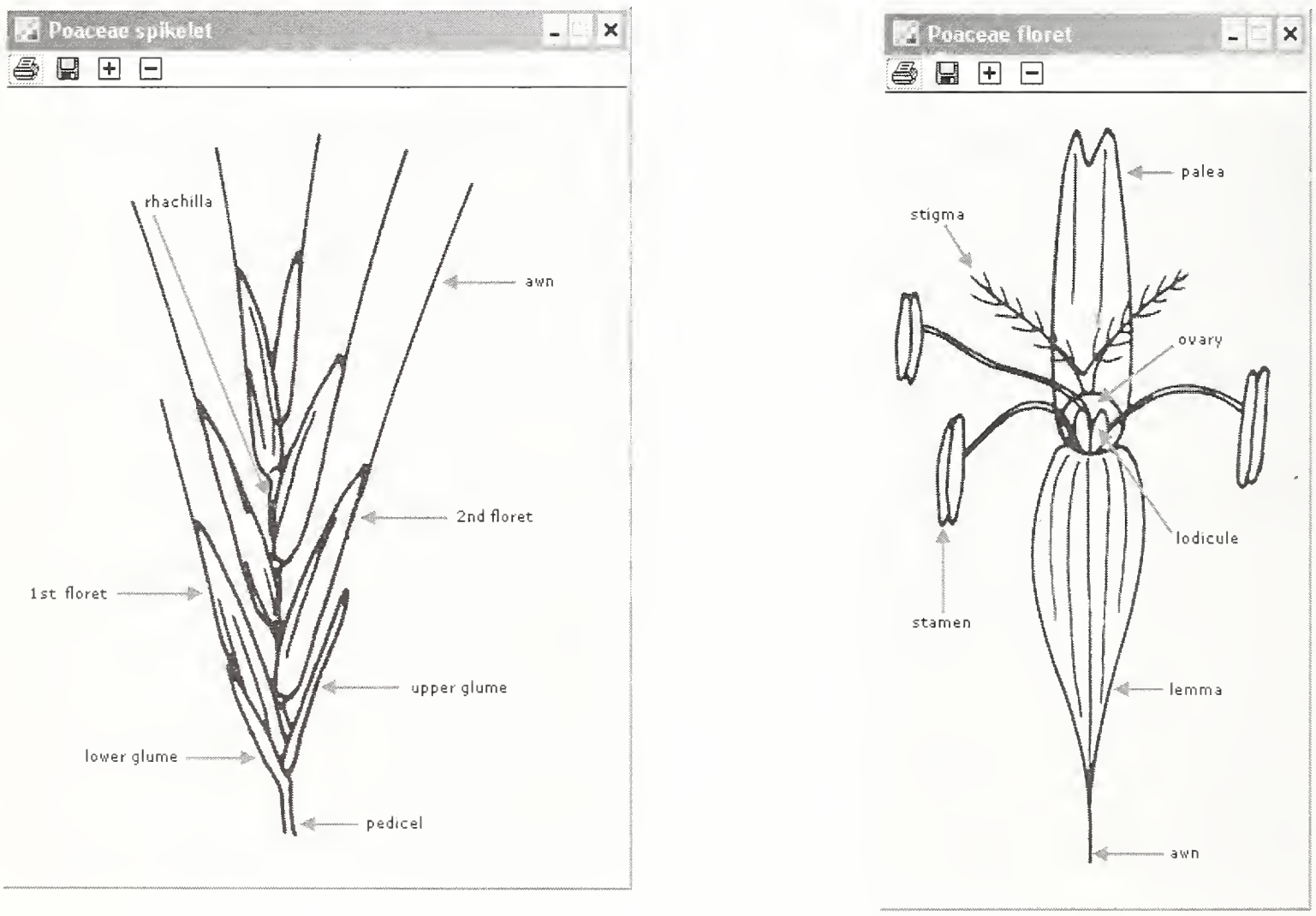

Figure 2. Pages from Stace (2004) showing glossary illustrations linked to choices in fig. 1.

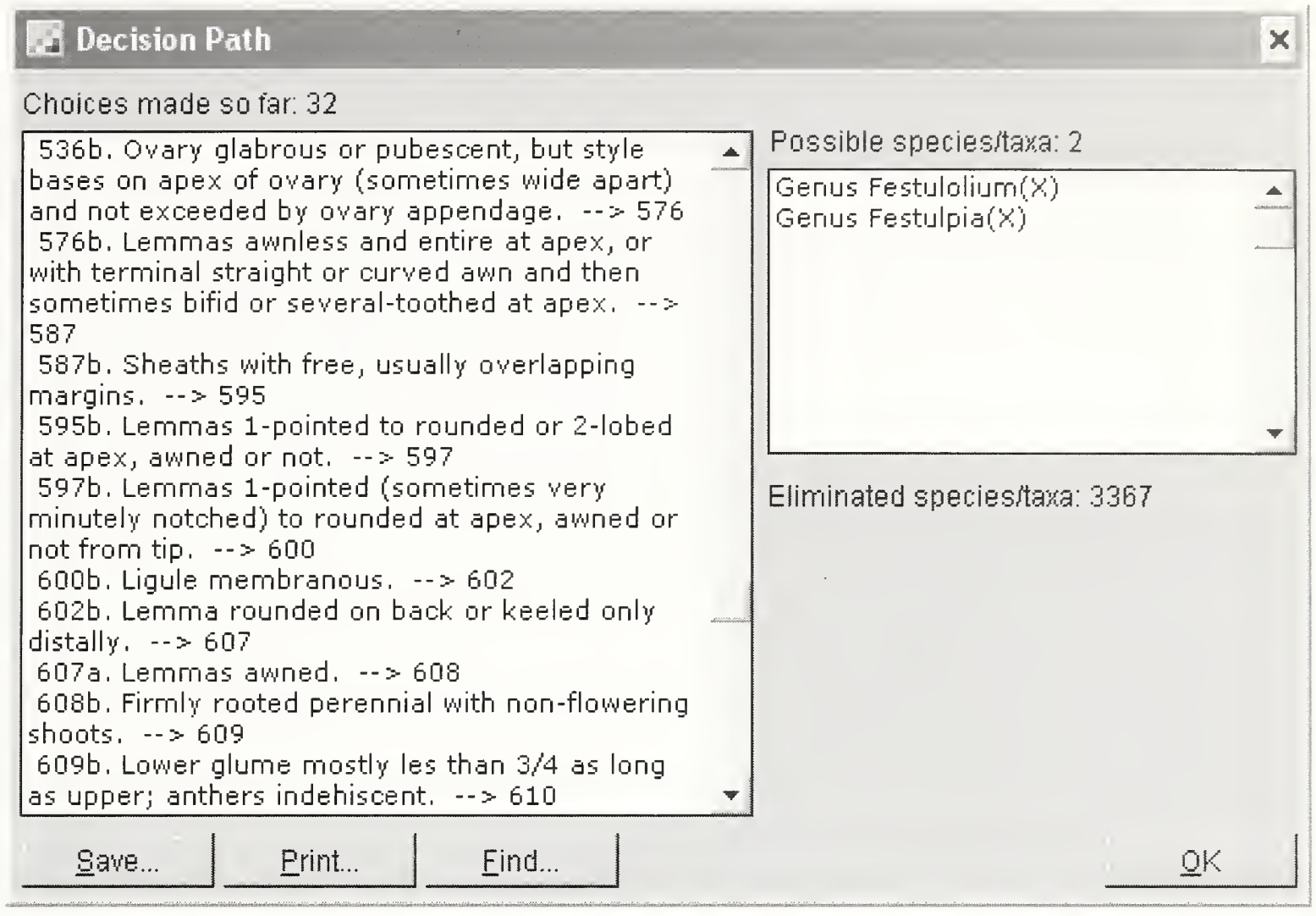

Figure 3. Page from Stace (2004) showing list of decisions already made and taxa remaining.

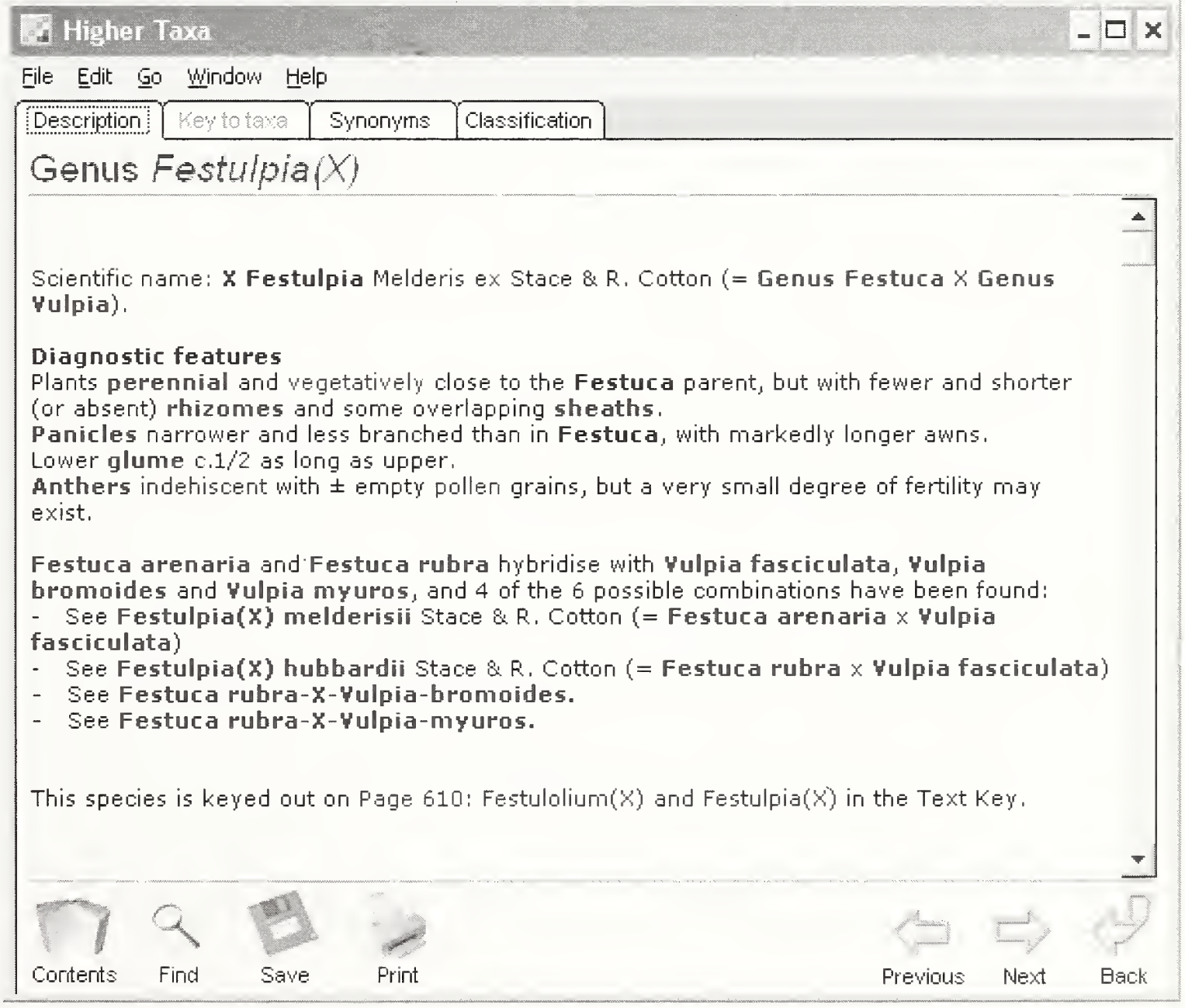

Figure 4. Page from Stace (2004) showing a Genus page with diagnostic features and links to various other pages.

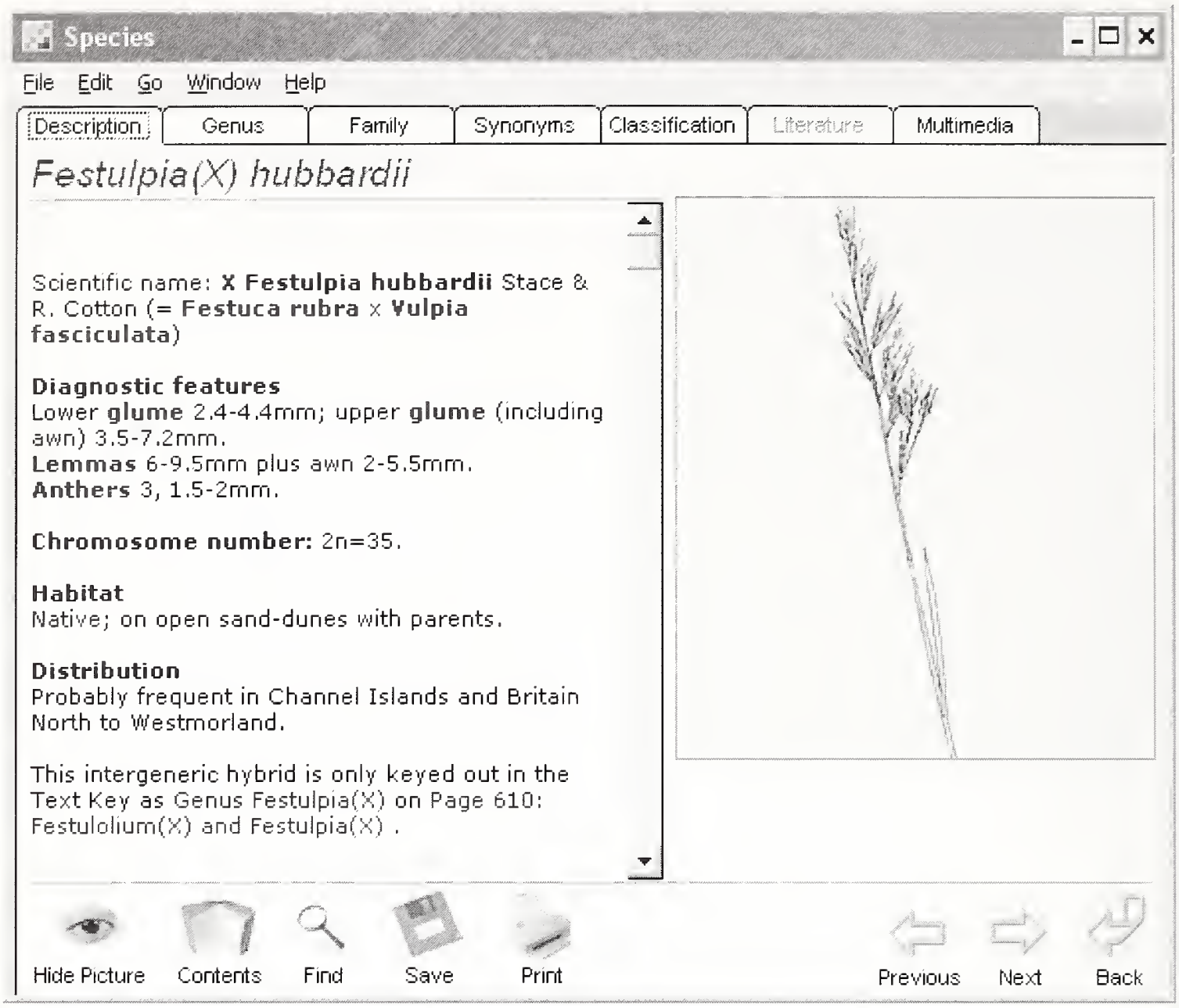

Figure 5. Page from Stace (2004) showing a Species page with illustration and links to various other pages, including more illustrations.

Although this sorting program must still be improved considerably, its advantage is that the user does not need much botanical knowledge. This is an important factor, as botany now plays a very minor part in education programs at schools and at home. If we succeed in making our floras as easy to handle as possible, we will get again the attention of the younger generations. Then the electronic flora will become a tool to widen the potential pool of plant observers, and to rejuvenate this group. This is necessary for the public awareness of the ongoing changes in the wild flora and the effects of this for the whole environment.

REFERENCES

ANDERBERG, A. & A.-L. (1998). *Den virtuella floran*: see http://linnaeus.nrm.se/flora/

LAUBER, K. & WAGNER, G. (1997). *Flora der Schweiz*. Haupt, Berne.

MEIJDEN, R. VAN DER (1999). *Heukels' Interactieve Flora van Nederland*. ETI, Amsterdam.

PETROVA, A., ASOV, B., VASILEV, R., DIMITROV, D., & JOTOV, G., (2002). *Flora Bulgarica*: see http://bsbcp.inet.bg/flora

SEYBOLD, S. (2001). *Schmeil-Fitschen Interaktiv*. Quelle&Meyer, Wiebelsheim.

STACE, C.A. (2004). *The Interactive Flora of the British Isles*. ETI, Amsterdam.

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New field botanists for the future

FRANKLYN PERRING†

Clive Stace was a precocious field botanist who contributed to the first Maps Scheme as a teen-aged boy in the mid-1950s, significantly encouraged by his biology master and the local natural history society in Tunbridge Wells and by the nearby Francis Rose. And he was not alone – other youngsters, notably Raymond Harley at Harrow and David Allen at Rugby, were similarly encouraged to high quality work. But where are such young people today?

Today very few biology teachers have the taxonomic skills, or the realisation of the need for field botanists, for them to be able or willing to promote it as a hobby, let alone as a career. Yet it is clear that there never was a time when there was so much demand for trained field botanists and never a time when there were so few available. To meet this gap the BSBI is working with many others to produce materials, literature and courses to make opportunities for careers in field botany widely known. These projects include:

SPOTLIGHT ON PLANTS

32 free places offered annually to second-year sixth formers in collaboration with the Field Studies Council (FSC).

TREES AND SHRUBS WEBSITE

Developed by BSBI/SAPS (Science and Plants for Schools), this is now being expanded to cover winter trees and twigs (www-saps.plantsci.cam.ac.uk/trees/home.html) (see fig. 2, p. 9).

BOTANIC GARDENS LEAFLET

Information on how to get to know British Plants, available via the Botanic Gardens Education Network (BGEN) in all Botanic Gardens.

CERTIFIED COURSES IN BIOLOGICAL RECORDING AND PLANT IDENTIFICATION

Birmingham University has a range of courses, starting with the part time field based certificate in *Biological recording and species identification*. An identification masterclass for experienced botanists to study with one or two expert tutors for five days to get their ID skills to a high enough level to sit a mock IDQ administered by the Natural History Museum. Finally, there is the part-time MSc on Biological Recording.

Leicester University is also active in this field and offers a certificate in *Applied plant studies: wild and garden plants*.

The BSBI will be working with the NHM to develop a range of IDQ qualifications for vascular plants.

CAREERS IN FIELD BOTANY

A two-page leaflet, *Why choose Plant Science as a Career*?, has been prepared and is being distributed to schools and careers agencies.

LEARNING ABOUT PLANTS

A very productive partnership with the Wildlife Trusts which has resulted in many local one-day courses and has generated a 4-page leaflet to give to all actual or potential students.

Editors' note – The above notes were prepared by Franklyn Perring when he was still intending to attend the meeting shortly before his untimely death. They were delivered by Philip Oswald. It should be emphasised that Franklyn was deeply involved with some of the above schemes; he was running field days at FSC centres and for the Leicester University course up to his final year, and the excellent Trees and Shrubs Website for school pupils is largely due to him.

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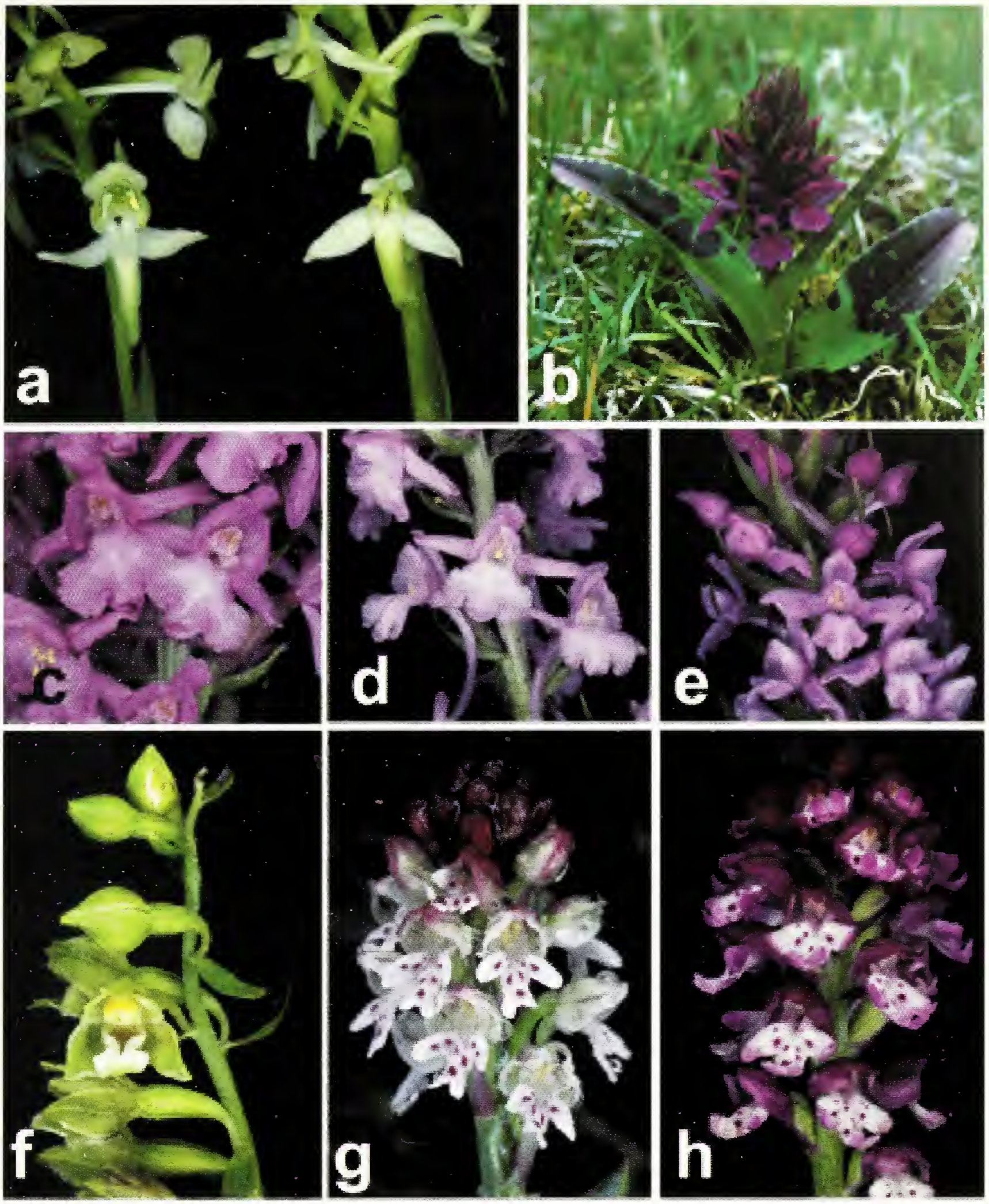

PLATE 1. (a) *Platanthera chlorantha* (left) versus *P. bifolia* (right) from Skye; (b) *Dactylorhiza ebudensis*, a recently evolved species confined to North Uist; (c–e) Marshland (c), grassland (d) and heathland (e) species of *Gymnadenia*; (f) *Epipactis sancta*, a recently evolved species confined to Lindisfarne; (g–h) Early-flowering (g) versus late-flowering (h) *Neotinea ustulata* in southern England (p. 93-102).

PLATE 2a. *Arabidopsis suecica* on a railway in Funbo, Uppland, Sweden.
Photo © B. Jonsell (p. 124).

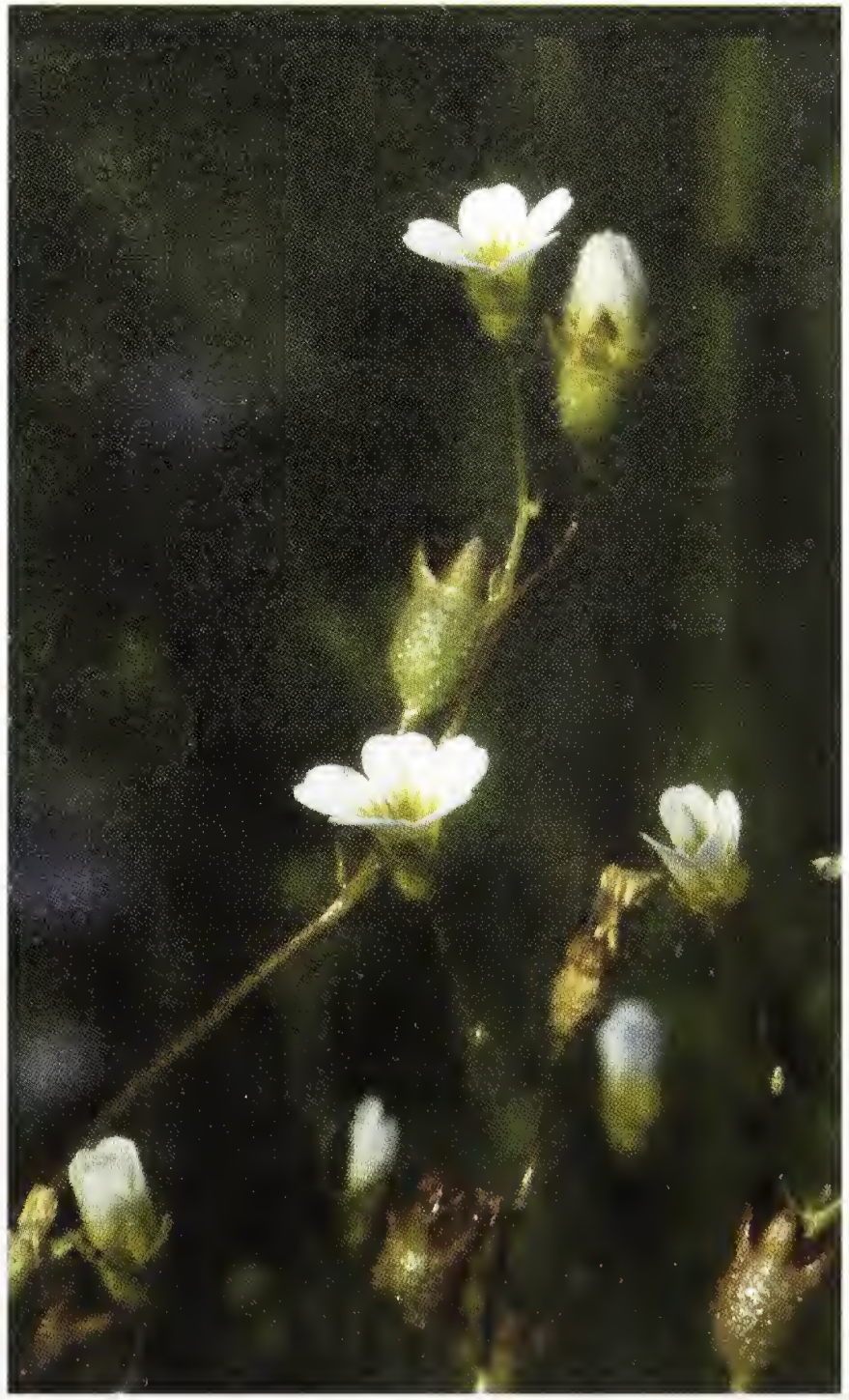

PLATE 2b. *Saxifraga osloënsis,* Östhammar, Uppland, Sweden.
Photo © B. Jonsell (p. 125).

PLATE 2c. *Deschampsia bottnica* on the Baltic coast at Hållnäs, Uppland, Sweden.
Photo © B. Jonsell (p. 129).

PLATE 3. Clive relishes the sight of *Sesleria caerulea* at it's southernmost outpost in Monk's Dale, Derbyshire (p. 2).

PLATE 4. John Richards identifying a yellow composite at Monk's Dale, Derbyshire (p. 2).

PLATE 5. The party at Houndkirk Moor, Derbyshire, site of one of the most southerly stations of *Trientalis europea* in the British Isles (p. 2).

This volume contains papers from the 'Current Taxonomic Research Work on the British & European Flora' meeting organised by the BSBI to commemorate Clive Stace's retirement from the University of Leicester. The papers closely mirror his particular interests, but still form a cohesive whole in the area of Floristics and Phylogeny. Papers range from regional Floras, through new approaches to Keys and electronic Floras to more detailed studies of particular plant groups, often involving DNA based techniques. The book takes an optimistic look at the future directions of plant taxonomy and will be of value to anyone interested in the European flora.

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