## Technology Transfer: Applications of Electronic Technology in Ecology and Entomology for Species Identification

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Abstract Recent advances in electronics and computing technology are leading to new applications in biology, ecology and conservation; one particular research area, computer-aided taxonomy, is only becoming a reality because of these advances. Computer-aided taxonomy encompasses automatic species identification, computer-based identification keys and taxonomic methods such as cladistics. Whilst there is a reasonable research effort being put into the development of computer identification keys for economically important groups, there is relatively little research on automated identification of species. Applications involving automated identification are diverse and include insect counting and sorting, pest monitoring and biodiversity assessment. Accordingly, this paper concentrates on automated identification research and describes recent and ongoing work at Hull University and elsewhere on two topics-bioacoustic identification of insects and birds, and image processing applications for discrimination of quarantine species of fungi and insects. The paper is based on the Open Lecture given at the Natural History Museum and Institute, Chiba, Japan on March 21, 1998.

**Key words:** computer-aided taxonomy, automated species identification and recognition, bioacoustics, image processing, computer applications, entomology, ecology.

In recent years, the growth in the electronic and computing industries has been remarkable, mainly due to the development of high speed, low cost microprocessors and memory. The advent of computers operating at 300 MHz or higher, with hundred's of megabytes of memory have led to new applications unheard of only a few years ago. In biology and ecology, there are now many possibilities for the development of research tools, commercial instrumentation and software packages for biological analysis. It is also becoming increasingly apparent that these new applications can only be properly designed if biologists have a modest knowledge of engineering and vice versa. Indeed, it is the author's belief that the only way forward is to increase multidisciplinary teaching and research in universities and colleges. It is from the multidisciplinary background of the members of the Control and Intelligent Systems Engineering Research Group, a merger of the Control Research Group and the Environmental Electronics Research

Group, at Hull University that the research projects described in this paper have arisen. The paper describes a number of projects that fall under the heading of computeraided taxonomy (CAT), a new term which was first defined at the inaugural meeting of the BioNET International Group for Computer-aided Taxonomy (BIGCAT) in Cardiff, Wales in July 1997 (Chesmore, 1998b). CAT is defined as the application of any computer or computer technique for taxonomic purposes.

CAT can be divided into 3 groups: automated identification systems, identification keys and software techniques for taxonomy such as cladistics. It has great potential for aiding species identification, especially during biodiversity studies where there may be many species. The quality of such surveys is dependent on the accuracy of the identification process which may be difficult and time-consuming. Edwards and Morse (1995) give a detailed account of the development of computer-aided identification mainly in keybased systems. It is beyond the scope of this paper to discuss key systems which are generally regarded to be well developed (see for example, Dallwitz *et al.*, 1998 and Pankhurst, 1991). Instead, the paper concentrates on more recent work involving automated and semi-automated species identification. More detailed discussions of CAT can be found in (Chesmore, 1997a, b; Chesmore and Morse, 1997).

#### **Automated Species Identification**

Before considering automated identification applications it is important to make a distinction between "identification" as described here and the term used for classification which is in wide use in taxonomy. In the applications described in this paper, "identification" is used in the context of associating an unknown signal (acoustic signals, images, etc.) with one in a pre-defined set of classes (species or groups). The identification system is trained with signals from each class prior to use. There is an ongoing debate as to whether "identification", "recognition" or "classification" should be used; it is the author's experience from meetings such as the Systematics Association Biennial Conference (Chesmore and Morse, 1997) and Fifth Workshop of the ESF Network in Systematic Biology on New Directions in Systematics (Chesmore, 1997b) that "identification" is the preferred term in Europe and that "classification" is to be restricted to taxonomy even though it is used commonly in engineering applications.

The concept of automated identification of species has received relatively little attention until recently; Table 1 shows some examples of current and past research areas. The techniques employed generally fall into 2 broad categories—acoustics and image processing, with a few miscellaneous methods involving flow cytometry for algae and phytoplankton (Balfoort *et al.*, 1992; Boddy *et al.*, 1994), and radar for aerial insect migration (Smith *et al.*, 1993).

Species identification by electronic means

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 Species/Group	Sensor(s)	Classification Method(s)
 Fish species	Active acoustics (sonar)	PDF, cluster analysis, ANN
 Orthoptera	Passive acoustics	TDSC+ANN
Amphibia	Passive acoustics	FFT+machine learning
 Birds	Passive acoustics	FFT, LPC, TDSC+ANN
 Mosquito	Passive acoustics	Wingbeat frequency
Flying insects	Radar	Scatter
	Infra-red Doppler	Wing beat frequency
 Lepidoptera	Monochrome & colour image	Colour discrimination
 Phytoplankton	Flow cytometry	ANN
	Monochrome image	Linear discrimination, ANN
 Hymenoptera	Monochrome image	Wing venation; PCA
 Leaf-miners	Monochrome & colour image	ANN
Fungal spores	Monochrome image	ANN, shape discrimination
Plants, weed species	Monochrome & colour image	FFT, colour discrimination
 Nanofossils	Scanning electron microscope	General image processing
Pollen	Scanning electron microscope	Texture analysis

 Table 1. Examples of species identification systems.

Notes: ANN, artificial neural network; PDF, probability density function; TDSC, time domain signal coding; FFT, fast Fourier transform; LPC, linear predictive coding.

can be considered to be an application of general pattern recognition in which an unknown (specimen) is placed into one of a number of possible classes depending on features extracted from measurements on the species. Pattern recognition has many applications ranging from handwriting recognition to speech analysis and identification of faults in machinery (condition monitoring). Automated species identification is very similar to many of these applications. Two main levels of automation can be identified—partially and fully automated as described below:

- a) Fully Automated. Complete identification without user interaction; this requires highly reliable identification with a very low (ideally zero) probability of misclassification.
- b) Semi-automated. This category is perhaps more realistic than a) as it allows prior sorting into higher taxonomic categories and presents the user with data for further manual identification if required. It is a relaxation of fully automated identification and is therefore more likely to be feasible in the short term.

It is anticipated that semi-automated systems will be the most viable as they allow the user to perform or verify the final identification. This is considered to be more acceptable in the short term as there is a tendency for humans to mistrust computers or consider them as a threat which may result in a possible impediment to CAT. It is therefore expected that semi-automated systems will play an important role in validation of techniques and in obtaining general acceptance of automation. In addition, such systems must not be seen as replacements for trained taxonomists but as identification aids.

Applications of automated identification systems are diverse and include insect counting, biodiversity assessment, ecological monitoring and detection/identification of pests. Each area is discussed in more detail below:

a) Insect Counting. Little research has been carried out in this potentially important area. Gonzales (1986) developed a pilot image processing system for identifying insects from agricultural surveys in an effort to speed up the often time consuming sorting process. It has been suggested that systems of this nature could aid considerably in sorting from large catches even if the sorting process only identifies to order or genus. Such pre-sorting could reduce the identification time by an order of magnitude. This also links to biodiversity assessment and pest monitoring (see below).

- b) Biodiversity Assessment. Systematics Agenda 2000 (1994) has stated that biodiversity inventories must be carried out for as many habitats as possible. However, increasing destruction of many habitats has led to the necessity for developing more rapid biodiversity assessment in an attempt to identify habitats and areas rich in biodiversity. Riede (1993) suggested that since many rainforest species produce sounds, it may be possible to use acoustic analysis for monitoring fauna. Riede used Orthoptera for more rapid biodiversity estimation in a tropical lowland forest in Ecuador and Oba (1994, 1995) used bird song as a measure of the "natural sound diversity" in Japan. Until very recently, it has only been possible to identify species manually from recordings which is both costly and time consuming; the development of automated identification systems will speed up the process and lead to continuous real-time monitoring.
- c) Ecological Monitoring. Potential applications for ecological monitoring are diverse and include recording the occurrence of call types and correlating with environmental conditions, long term continuous monitoring and determination of bird species for species-specific bird strike avoidance (bird scarers) in airports. It is also theoretically possible to identify and monitor individuals in populations of some taxa (e.g. birds).
- d) Pest Monitoring. Many animal pests, particularly insects, can be detected by their sound production. In the USA, Shuman *et al.* (1993) used acoustics for detecting beetle larvae in rice grains. Hagstrum *et al.* (1990) used similar techniques for monitoring of *Rhizopertha dominica* (Coleoptera: Bostrichidae) in wheat

kernels. It is theoretically possible to detect and, more importantly, identify many different insect and animal pests in a variety of agricultural and horticultural environments although very little work has been done to date.

#### **Identification Using Bioacoustics**

Many insect, bird and animal species produce sounds either deliberately for communications or as a byproduct of movement (flying, eating, walking, etc.). In many cases, such sounds can be used to detect the presence of animals or, if sufficiently distinctive, identify species. The majority of acoustic applications are passive, i.e. they rely on calls and sounds produced by animals. The majority of research to date has been on the identification of birds in general (McBraith and Card, 1995; Anderson et al., 1996), nocturnal migrant birds (Mills, 1995), frogs and other amphibia (Taylor et al., 1996) and insects (Chesmore et al., 1998; Chesmore and Nellenbach, 1997). Applications involving detection rather than identification are less complex and have mainly been for stored grain insect pests (Shuman et al., 1993; Shuman et al., 1997; Weaver et al., 1997; Anderson et al., 1996).

An alternative to passive listening is active sonar which relies on scatter from transmitted sound, often ultrasound. Underwater sonar applications are currently restricted to identification of fish species in shoals (Simmonds *et al.*, 1996; Scalabrin *et al.*, 1996) and zooplankton classification (Martin *et al.*, 1996). The latter research project does not identify to species but determines approximate groupings according to acoustic spectral reflection characteristics.

# Development of the Intelligent Bioacoustic Identification System (IBIS)

IBIS is a multipurpose testbed for automated bioacoustic identification applications. It is based on a technique known as time encoded speech (TES) which was developed in the 1970's by King (King and Gosling, 1978) as a purely time domain approach to the compression of speech for digital transmission. It has subsequently been used in a number of applications including acoustic



Fig. 1. Definition of a TDSC epoch.

condition monitoring of machinery (Lucking et al., 1994) and heart sound analysis and defect identification (Swarbrick and Chesmore,1998). TES is now termed time domain signal coding (TDSC) by the author (Chesmore, 1998c) since the term is more general than the original application. TDSC characterises any bandlimited signal by its shape between successive real zeros (termed an epoch); generally, this shape is taken between actual zero-crossings. Each epoch is described in terms of its duration in samples (D) and shape (S) usually taken as the number of minima or signal energy as indicated in Fig. 1 which shows a 10 sample epoch with 2 minima (D=10, S=2). The number of possible D-S combinations (symbols) is termed the natural alphabet which can often be nonlinearly mapped onto a smaller symbol set to give signal compression. In the original speech application, the coded symbols were transmitted and used to regenerate the speech signal at the receiver thus providing digital speech transmission at substantially reduced data rates.

TDSC can be described as the concatenation of a signal's D–S symbols, i.e. it produces a sequential stream of symbols and one analysis method is to examine the occurrence of pairs of symbols over time to give a histogram, A, which describes the number or proportion of symbols i and j occurring in succession, i.e. the number of times i is followed by j by a lag L. A 2-dimensional histogram, the A-matrix, can be formed, expressed mathematically as:

$$a_{ij} = \frac{1}{(N-L)} \sum_{n=L+1}^{m=N} x_{ij}(n)$$
  
here  $L = \log$ 

w

$$x_{ij}(n) = 1$$
 if  $t(n) = i$  and  $t(n-L) = j$   
(0 otherwise)  
 $t(n) = n^{\text{th}}$  TES symbol

This fixed size histogram with timeinvariant dimensions is the feature set used for classification purposes. The entry at position (i, j) represents the number (percentage) of occurrences of the TDSC symbol pair i and j where j is delayed relative to the first (in epochs). In this application, a lag of 1 epoch is used; multiple lags may also be employed giving rise to multi-dimensional matrices. The A-matrix is independent of any gain factors if the input signal has no dc component and is therefore insensitive to relative energies of different segments of the signal.

Each sound (call, syllable, etc.) is transformed into a single A-matrix which forms the basic feature for pattern classification using an artificial neural network (ANN). ANNs are now widely used in many classification and identification problems as they can be trained, are good at handling fuzzy and disparate data and are able to perform non-linear discrimination. There are many forms of ANN which can be divided into supervised (requires training) and unsupervised classification (no training). The majority of classification methods used in IBIS are based on multilayer perceptrons (MLP) using backpropagation for training. More recently, self-organising features maps have been investigated with some success.

All software has been developed using Matlab, a generic mathematical programming language optimised for matrix manipulation. Matlab has been chosen for two reasons—it is simple to use and a wide range of toolboxes are available, in this case the neural networks toolbox. Matlab runs on any 486 computer (or better) with at least 4 Mbytes of RAM and a hard disc, and is capable of running on portable PCs. The sounds are sampled at 44 kHz, 16-bits per sample using a program called Goldwave V3.03 via the line input of a Soundblaster card.

## **Bioacoustic Identification Examples**

To date, 2 different test groups have been selected—25 species British Orthoptera and

ID Code	Latin Name	English Name
OR01	Meconema thalassinum	Oak Bush-cricket
OR02	Tettigonia viridissima	Great Green Bush-cricket
OR03	Decticus verrucivorus	Wart-biter
OR04	Pholidoptera griseoaptera	Dark Bush-cricket
OR05	Platycleis albopunctata	Grey Bush-cricket
OR06	Metrioptera brachyptera	Bog Bush-cricket
OR07	Metrioptera roeselii	Roesel's Bush-cricket
OR08	Conocephalus discolor	Long-winged Cone-head
OR09	Conocephalus dorsalis	Short-winged Cone-head
OR10	Acheta domesticus	House-cricket
OR11	Gryllus campestris	Field-cricket
OR12	Nemobius sylvestris	Wood-cricket
OR13	Gryllotalpa gryllotalpa	Mole-cricket
OR14	Stethophyma grossum	Large Marsh Grasshopper
OR15	Stenobothrus lineatus	Stripe-winged Grasshopper
OR16	Stenobothrus stigmaticus	Lesser Mottled Grasshopper
OR17	Omocestus rufipes	Woodland Grasshopper
OR18	Omocestus viridulus	Common Green Grasshopper
OR19	Chorthippus brunneus	Field Grasshopper
OR20	Chorthippus vagans	Heath Grasshopper
OR21	Chorthippus parallelus	Meadow Grasshopper
OR22	Chorthippus albomarginatus	Lesser Marsh Grasshopper
OR23	Euchorthippus pulvinatus	Jersey Grasshopper
OR24	Gomophocerippus rufus	Rufous Grasshopper
OR25	Myrmeleotettix maculatus	Mottled Grasshopper

 Table 2.
 List of British Orthoptera species for IBIS tests.

10 species of bird occurring in Japan. Results for each test group will be considered separately.

1. Results for British Orthoptera

Table 2 lists the 25 species used in 1 set of tests. The sounds were derived from a



Fig. 2. Gryllus campestris (OR11): A, Time domain waveform; B, Average scaled A-matrix.



Fig. 3. Chorthippus albomarginatus (OR22): A, Time domain waveform; B, Average scaled A-matrix.



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Fig. 4. Meconema thalassinum (OR01): A, Time domain waveform; B, Average scaled A-matrix.

widely available audio cassette (Burton and Ragge, 1987) available as an accompaniment to The Grasshoppers and Allied Insects of Great Britain and Ireland (Marshall and Haes, 1988) and digitised as previously described. TDSC symbols for up to 2 seconds of sound for each species. The subsequent A-matrices were used to train a single layer Perceptron consisting of 784 inputs (1 for each location in the A-matrix) and 25 output neurons, 1 for each species. Figs. 2, 3 and 4 show time plots and A-matrices for 3 species-Gryllus campestris (OR11), Chorthippus albomarginatus (OR22) and Meconema thalassinum (OR01). The single layer Perceptron was developed by Rosenblatt in the 1960's (Rosenblatt, 1962) and is a competitive learning network that performs n-dimensional discriminant analysis. The results suggest that TES is a good pre-processor providing wide separation of sounds which would have similar spectra. This is evident when the example A-matrices in Fig. 2B, 3B and 4B are examined. The differences between species are obvious, especially for M. thalassinum which has predominantly low frequencies compared with the other 2 species.

Once trained, the system was tested with new sounds; Table 3 shows identification results with various levels of added noise to simulate response to poorer conditions. Each entry is an average of 1000 normally distributed random A-matrices (zero mean, unity variance) added to the A-matrices which simulates Gaussian white noise over the whole frequency spectrum. It is evident from Table 3 that identification is very high (99-100%) under low noise conditions with the exception of Metrioptera brachyptera (OR06) and that mis-identification is zero until 30% noise is added. The latter is a fundamental requirement for an automated system.

However, it is important to note that some species exhibit a very rapid decline in identification accuracy (OR01—Meconema thalassinum and OR13—Gryllotalpa gryllotalpa). Both species have characteristically low dominant frequencies and this may contribute to confusion. However, it is known that the former species produces substratebased sounds and would not be detected in a bioacoustic survey. Further work on determining the reasons needs to be carried out.

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ID Code	Noise Level (%)								
ID Code	1	3	5	10	20	30	40	50	
OR01	99.9	83.5	54.2	24.3	8.7	5.3	4.7	4.3	
OR02	100	100	100	100	96.7	89.3	82.3	76.8	
OR03	100	99.6	94.8	76.8	63.8	60.6	58.6	53.6	
OR04	100	100	100	99.9	94.1	86.8	79.3	72.0	
OR05	100	100	99.4	90.1	71.1	66.9	59.0	57.9	
OR06	85.6	65.5	58.8	54.3	54.3	52.0	52.0	47.9	
NR07	100	100	99.9	94.2	65.0	45.1	29.1	21.6	
OR08	100	100	100	100	99.1	95.1	87.4	79.0	
OR09	100	100	100	100	100	99.7	98.4	95.5	
OR10	100	100	100	100	99.4	94.8	84.5	76.0	
OR11	100	100	100	100	100	100	100	100	
OR12	100	100	100	100	99.1	93.8	84.1	73.8	
OR13	100	84.9	63.7	34.2	16.5	9.3	7.6	6.0	
OR14	100	100	100	100	93.4	85.7	76.1	73.6	
OR15	100	100	99.7	89.3	74.9	61.5	58.9	52.2	
OR16	100	99.9	98.0	84.9	68.0	63.8	59.2	59.4	
OR17	100	86.4	74.4	63.4	58.2	52.8	50.2	45.8	
OR18	100	98.1	86.7	59.7	47.0	38.1	35.4	32.8	
OR19	100	100	99.9	95.6	80.7	71.5	62.8	62.9	
OR20	100	100	99.9	91.3	77.1	67.7	63.7	60.5	
OR21	100	100	100	100	99.8	97.5	92.7	81.8	
OR22	99.8	86.1	74.1	62.3	54.5	56.4	53.5	54.5	
OR23	100	100	100	99.7	94.1	84.6	78.5	71.9	
OR24	100	99.6	92.0	65.8	48.9	39.6	36.7	30.6	
OR25	100	100	100	100	100	99.8	98.9	96.6	
Mean positive ident. (%)	99.4	96.1	91.8	83.4	74.6	68.7	63.7	59.5	

Table 3. Orthoptera species identification accuracy with added noise. For ID code, see Table 2.

Table 4.	Species	of Japanese	bird	used	in	IBIS	tests.
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ID Code	Latin Name	English Name			
JB01	Acrocephalus arundinaceus	Great Reed Warbler			
JB02	Cuculus canorus	Common Cuckoo			
JB03	Cettia diphone	Bush Warbler			
JB04	Cuculus poliocephalus	Little Cuckoo			
JB05	Emberiza variabilis	Gray Bunting			
JB06	Ficedula narcissina	Narcissus Flycatcher			
JB07	Megalurus pryeri	Japanese Marsh Warbler			
JB08	Parus major	Great Tit			
JB09	Phylloscopus tenellipes	Pale-legged Willow Warbler			
JB10	Turdus chrysolaus	Brown Thrush			

Variations in classifier using MLPs and expert system identification have been assessed, all with reasonable results (Chesmore *et al.*, 1998; Chesmore, 1997c, 1998c).

### 2. Results for Birds in Japan

During the author's visit to Japan in March 1998, IBIS was tested on 10 species of bird (Table 4) which occur in woodland and grassland in Japan. The same approach was employed but the classifier was a MLP with 784 inputs (as before), 40 neurons in the hidden layer and 10 outputs (1 for each species). Sounds for training were obtained from CD (Kabaya and Matsuda, 1996a, b, c) and results were very encouraging. Table 5 gives the

	JB01	JB02	JB03	JB04	JB05	JB06	JB07	JB08	JB09	JB10
JB01	1	0	0	0	0	0	0	0	0	0
JB02	0	1	0	0	0	0	0	0	0	0
JB03	0	0	1	0	0	0	0	0	0	0
JB04	0	0	0	1	0	0	0	0	0	0
JB05	0	0	0	0	1	0	0	0	0	0
JB06	0	0	0	0	0	1	0	0	0	0
JB07	0	0	0	0	0	0	1	0	0	0
JB08	0	0	0	0	0	0	0	1	0	0
JB09	0	0	0	0	0	0	0	0	1	0
JB10	0	0	0	0	0	0	0	0	0	1

Table 5. Preliminary results for bird identification matrix. For ID code, see Table 4.



**Fig. 5.** *Cuculus poliocephalus*: A, Time domain waveform of the sound for training; B, Time domain waveform under natural conditions (woodland); C, A-matrix for the sound for training; D, A-matrix under natural conditions (woodland).

results in the form of a confusion matrix. It is evident that the recognition accuracy for good quality sounds is 100% with no misidentification. The system was tested further by extracting a single call of *Cuculus poliocephalus* from a woodland recording by Dr Oba from the Natural History Museum and Institute, Chiba (CBM Acc. No. 043–0086) and presenting this to the system. Fig. 5A shows the time signal under good conditions, 5B the time signal under natural conditions, 5C the A-matrix for the bird under good conditions and 5D the A-matrix for the bird under natural conditions. Under these conditions the species was recognised with 86% accuracy.

Again, the research is still in its infancy and much work needs to be done. One particular area of research which is considered essential is to preserve the time structure of the sounds since bird (and higher animals) song is complex in both the time and frequency domains. Work at Hull is currently investigating the separation of individual syllables and applying syntactic pattern recognition techniques.

## Image Processing Applications

Image processing is the second major sensor system for species identification. It is possible to use monochrome or colour images captured digitally, scanned from photographs or from scanning electron microscopes. It is evident from Table 1 that the range of applications is wider than for bioacoustics as image processing is more generic. For the purposes of this paper, it is convenient to divide the applications into species identification or discrimination and morphological analysis of individuals within populations.

Species identification research can be further sub-divided into categories describing the final potential application such as biodiversity assessment, discrimination between closely similar quarantine species, agricultural applications, paleobiology and paleobotany. Mention has already been made about image-based insect counting which aims to speed up sorting; other applications include identification of braconid hymenoptera (Weeks *et al.*, 1997; Weeks and Gaston, 1997) and solitary bees by their wing venation, leafhopper species (Dietrich and Pooley, 1994), blue-green algae (Thiel, 1994) and cyanobacteria. Discrimination of closely similar species for quarantine purposes will be described in detail later in the paper. Agricultural applications have concentrated on real-time identification of weed species for herbicide placement, part of the concepts of integrated crop management (ICM) and integrated pest management (IPM). Recent research has used fractals and Fourier analysis (Critten, 1996) and colour chromaticity (Shulin and Runtz, 1995). A related application using spectral reflectance to discriminate between plants and soil is described in Vrindts and De Baerdemaeker (1997). It should be noted that the latter project does not use image processing but near IR diffuse spectral reflection. Paleobotany research has been on identification of pollen from sediment cores in lakes to recreate environmental histories (Langford et al., 1990); no other research projects have been found to date.

Morphological analysis (measurements) using image processing is well established in many fields such as biomedicine (e.g., X-ray images) and engineering (e.g. robotics, fault diagnosis) but less well so in biology and even less in entomology. The main entomological applications are for Lepidoptera to measure genetic influences on wing patterns, for example Windig *et al.* (1994) used an image processing system for quantifying seasonal polyphenism in species of *Bicyclus* butterflies.

## Image Processing Applications at Hull

The image processing projects at Hull can be divided into two categories—morphological analysis of Lepidoptera and automatic discrimination of closely similar species. All the research has been carried out using custom software written in Visual C for Windows or Matlab. Matlab has the advantage of simplicity in prototyping algorithms but is interpreted and hence slow whereas C requires a detailed knowledge of programming but is very fast. Matlab also has a number of toolboxes such as image processing and artificial neural networks which speed up the development process. Images are derived



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Fig. 6. Image processed Biston betularia forewings.

from digital cameras attached to microscopes or in the case of Lepidoptera directly from a digital camera; live specimens can be photographed without harm using an electroimmobilisation unit developed at Hull (Chesmore and Monkman, 1994).

## Morphological Analysis

Morphological analysis of Lepidoptera has been attempted for a number of different applications including analysis of variation in British butterflies, quantitative analysis of melanism in *Biston betularia* for industrial melanism studies and analysis of the colour morphs of *Noctua pronuba*. One future application will be determination of fluctuating asymmetry in *Melanargia galathea*, a species of butterfly which occurs in sporadic populations in the Yorkshire Wolds not far from Hull. The first 2 applications will be discussed in more detail.

Quantitative Analysis of Melanism. Melanism is exhibited in a number of moth species in several countries in the World. Britain appears to have the highest number of species, the most notable being the Peppered moth, *Biston betularia* which has 2 common forms—f. *typica* which has a white background and many black spots, and f. carbonaria which is entirely black. F. carbonaria was thought to exist in very small proportions prior to the industrial revolution in Britain but became the predominant morph very rapidly after the onset of the revolution. In some heavy industrial localities such as Manchester, the proportion of f. carbonaria became 100% and remained so until the 1960's when a clean air act was introduced, reducing smoke pollution dramatically. Since then, the proportion of f. carbonaria has reduced in most localities as lichens have re-established. However, the traditional explanation of selective predation due to the light form being visible on dark, smoke covered tree trunks and hence being eaten, has some problems. In America, the same increases and decreases of a very closely related species have followed the same trend as in Britain but without the loss of any lichens. Also, B. betularia has an intermediate form, f. insularia, which is thought to be continuously variable between the 2 extremes. In addition, in 1 study in Helsinki using 2 closely related moth species (Oligia latruncula and O. strigilis) has further confused the situation in that O. strigilis has increased in melanic pro-

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Fig. 7. Example of continuous variation in Biston betularia.

portions while O. latruncula has decreased over the same period. Image processing has been used to measure the proportions of black and white in a forewing of B. betularia in order to quantify the amount of melanism (Chesmore and Yorke, 1997a, b; Chesmore, 1998a). This is achieved by detecting the wing edge, using thresholding to discriminate between the white and black pixels and then counting the number of black pixels. The result is normalised against wing area and expressed as a percentage. Fig. 6 shows 2 wings, 1 of each form, processed in this manner, together with estimates of "blackness". Fig. 7 shows how it is possible to give more detail using this technique instead of classifying "normal" or "melanic" (includes f. insularia). Research is ongoing and it is hoped that more extensive trials will take place in 1999.

Analysis of *Noctua pronuba* Forms. This moth is extremely common in Britain and can be a pest in gardens. It exhibits up to 7 named colour forms and is sexually dimorphic. Because of this and it's abundance, it was chosen as a test subject for image analysis with an emphasis on colour discrimination of forms. In Yorkshire only 4 forms are commonly found; these are f. *ochrea* (female), f. *rufa* (female), f. *innuba* (male) and f. *ochrea*-

brunnea (male), with the last being the commonest recorded at the trapping site (using a low power ultraviolet light trap). Images of detached forewings were obtained using a JVC colour CCD camera and digitised with a frame grabber installed in a Pentium computer. Each image was digitised to  $272 \times 320$ pixels, 24-bit colour. Each wing was digitised with a blue background which provided a strong contrasting colour to the wings and could be removed by simple thresholding of the blue image. In 1 study 58 features were extracted from each wing image, including mean, median, mode, standard deviation, Kurtosis, energy and entropy of the green (G), red (R) and blue (B) channels, R-G, B-G, R-B covariance and correlation, moments and edge densities (Chesmore et al., 1996). This number was reduced to 16 features describing each wing by considering the ranges of variability of the parameters and rejecting those with very small variability. The methods included principal components analysis, cluster analysis and unsupervised neural networks. In cluster analysis and neural networks, 5 clusters were selected to correspond to the 4 forms and a fifth as a "catch-all". Different colour forms can be distinguished successfully, f. ochrea being almost always classified perfectly. It should

also be noted that some entomologists consider several of the forms as continuously variable and the named forms are extremes; this is borne out to some degree in these results since in most cases, f. *ochreabrunnea* is spread across several groups.

## Automatic Discrimination of Closely Similar Species

This research is more recent than previously described work and there are fewer results. The two projects under way are both related to the early detection of quarantine species which are considered to be too dangerous (in terms of economy) to be allowed into Britain. The work is in association with Central Science Laboratory (CSL) located near York and one of its roles is to examine consignments of plants for import to assess the presence of pests. It is important to be able to detect the presence of pests as early and accurately as possible and this is carried out manually by experts. The taxa selected for the projects are Liriomyza spp (Diptera: Agromyzidae), a leafminer and *Colletotricrichum* spp, a fungus causing strawberry black spot.

Detection of *Liriomyza* spp. *Liriomyza trifolii* is an established pest in Britain on various horticultural crops such as tomato and chrysanthemum whereas *L. huidobrensis* is a quarantine pest and cannot be allowed into the country. It is therefore important to provide rapid and accurate identification, allowing for more specific targetting of pesticides and other control and eradication measures, minimising environmental damage, preventing further infestation and saving money. Identification is important but time consuming, often requiring identification of the larvae or waiting until they become adults. Image processing of the leaf mines may provide a solution. The ongoing project (Pether, 1998) is investigating whether it is possible to discriminate mines from each species; this is complicated by the fact that both species are polyphagous and the mine character is dependent on hostplant species. Fig. 8A shows a typical leaf mine and Fig. 8B shows how it is possible to discriminate between the leaf and mine using simple edge detection. Discrimination between C. acutatum and C. gloeosporioides. C. acutatum is an EC listed quarantine organism and is separated from C. gloeosporioides in part by examination of conidia which are morphologically similar. One of the key characters used is the shape of the spore's apex—described as acute for C. acutatum and obtuse for C. gloeosporioides. The aim of the project was to ascertain if image analysis could quantify this difference and reliably separate these species. Images were obtained from CSL and were derived



Fig. 8. Chrysanthemum: A, Leaf with leaf mine; B, Image processed leaf showing mine.

from microscope slides at 400 times magnification, digitised using a three chip colour camera (JVC-TK 1270E) and captured with a Snapper 24 frame grabber using computer software from Optimas (Seattle, USA). Initially, analysis was performed using Optimas (version 5.2) at CSL. Length (principal axis), breadth (minor axis) and area of conidia were measured and the data exported to Microsoft Excel where the area bounding the spore (length×breadth) and the area/bounding area were calculated. Images were then analysed using Matlab at Hull. A theoretical ellipse was generated from the principal and minor axes and then compared with the actual spore contour (outline). Secondly, the principal and minor axes were used to separate each spore into 4 segments and individual areas calculated. These were placed in a vector and multiplied by the Hadamard matrix to calculate the Hadamard-Walsh spectrum of the spore shape (related to symmetry properties). Results showed that there was a statistically significant difference between C. acutatum and C. gloeosporioides for the area/bounding area for conidial line drawings. Although, a similar trend was obtained for conidia from cultures it was not statistically significant. Comparison of spores with a theoretical ellipse failed to separate species reliably. The Hadamard function, however, showed greater promise. The vertical component for C. acutatum ranged from 1-2 whilst for C. gloeosporioides it was between 0-1, showing reasonable discrimination (Lane et al., 1998).

## Conclusions

The paper has given a brief outline of computer-aided taxonomy, concentrating on automated species identification. It is evident that there are many possible applications for automatic identification, perhaps the most important being the development of more rapid biodiversity assessment methods.

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## 技術移転:電子工学技術を生態学と昆虫学 における種識別へ適用する

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電子工学やコンピューター技術の最近の発達は、生 物学、生態学および環境保全に新しい適用の道を開い ている.なかでもこうした発展ゆえに際立った研究領 域として、コンピュータ依存型分類学が現実化しつつ ある. コンピュータ依存型分類学は自動種識別, コン ピュータを基礎とする検索表、および分岐学のような 分類法を包含する.一方で,経済的に重要な分類群の コンピュータを基礎とする検索表の発展には相応な研 究努力が投入されているが、種の自動識別には相対的 に僅かな研究しか行われていない. 自動識別が必要と される応用は多様で、昆虫の個体数のカウント、種類 の選別、有害昆虫のモニタリングおよび生物学的多様 性評価を含む. したがって、本稿は自動識別研究に着 目し、ハル大学他で近年および目下進行中の課題であ る, 昆虫と鳥類の生物音響学的識別と検疫菌類や昆虫 類の区別への画像解析の応用について記述する、本稿 は、1998年3月21日に千葉県立中央博物館で行わ れた公開講演会に基づくものである.