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The phylogeny of the endocrine system

by E. J. W. Barrington*

formerly University of Nottingham; 2 St. Margarets Drive, Alderton, Tewkesbury, Glocestershire GL20 8NY (England)

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The scope of phylogeny

Phylogeny, which is a representation of the evolutionary history of taxa, requires critical interpretation of biological diversity and of the relationship of this with the unity of organisation which underlies living systems. Some degree of personal judgement may be involved in particular cases, and so, because of this subjective element, phylogenetic propositions are always open to discussion in the light of new information. On the larger issues, however, and on many smaller ones as well, there is substantial agreement, founded on the rich classical resources of descriptive anatomy, which take account of large numbers of species, both living and extinct, and with embryo-

logical studies as a valuable supplement. The strength of these views derives not only from the wide range of material studied, but also from the possibility of evaluating the anatomical interrelationships of organs and systems which may be unduly influenced by convergence[10]. Further evidence is, of course, provided by physiological, biochemical and molecular studies, but in the euphoria engendered by recent advances in some of these areas it is easy to overlook their limitations: the paucity of species studied, ignorance of extinct forms, and the flexibility of functional organisation and its consequent susceptibility to opportunistic and convergent evolution. Caution is therefore demanded.

Nevertheless, it is fair to say that current views of the phylogeny of endocrine systems do conform in general with classical views of evolutionary history, yet the correspondence is not absolute, and our expectations are not always satisfied. For example, sequence studies[28] of the insulins of the chick, an alligator and two species of snakes support classical phylogenetic deductions drawn from living and fossil vertebrates, but it is salutary to be told that hormones do not always show such clear patterns; snakes, for example, possess an unusual gonadotropin, and perhaps only a single one[31].

However, limitations inherent in interpretations of endocrine phylogeny do not reduce them to a sterile pursuit. On the contrary, phylogenetic perspectives are essential if we are to analyse in sufficient depth such fundamental issues as the origin and diversification of biologically active molecules and their receptors, the exploitation of their hormonal potentialities, and their resulting contributions to complex and adaptively flexible regulatory systems. A brief review of some aspects of thyroid phylogeny will illustrate this argument.

Thyroidal phylogenesis

Thyroidal biosynthesis involves the uptake of iodide, its oxidation to reactive iodine by peroxidase, and the binding of this within thyroglobulin molecules into iodotyrosines which are then polymerised to form thyroxine and triiodothyronine. The gland is sharply restricted to vertebrates, yet organic binding of iodine, demonstrable by autoradiography, occurs in a number of invertebrates, in some of which small amounts of iodotyrosines and, less commonly, iodothyronines have been found. However, such binding is often associated with the formation of structural proteins, so that these iodinated products cannot be circulated and made available for general metabolism[2]. What, then, were the special circumstances that permitted the establishment of a thyroid gland so early in vertebrate phylogeny that it could be already well developed in the primitive jawless Agnatha (lampreys and hagfish)?

The generally accepted explanation is that the thyroid originated in a pharyngeal gland, the endostyle. This forms part of a unique ciliary feeding mechanism found in the protochordates (e.g. amphioxus and the ascidians), which are surviving representatives of early forerunners of the vertebrates. Peroxidase[13,27] and a thyroglobulin-like material[49] have been identified in the endostyle. So also have iodotyrosines and at least one iodothyronine[5], which can be absorbed in the intestine from the endostylar secretion, and thus be metabolically exploited. On this argument, phylogenetically ancient molecules have been introduced into the vertebrate endocrine system as a consequence of their production under uniquely favourable conditions in remote ancestors. It makes a plausible phylogenetic statement, but can we identify selective advantages that might have determined this proposed course of events?

The structural features required for thyroidal activity include 3,5,3′ substituents which need not be iodine or, indeed, halogens at all; halogen-free methyltyrosines, for example, have significant thyromimetic activity in tadpoles and rats. What, then, were the selective advantages of the iodothyronines? Frieden[17] points out that they would presumably have included the ready availability of iodine in the sea, where the chordate line arose. This advantage was, of course, lost when vertebrates passed through fresh water to the land, but by then it must have been too late to abandon an established molecular relationship. Other circumstances favouring thyroidal evolution would have included the availability of H_2O_2-peroxidase systems, and of ample proteases for the release of the iodothyronines from their peptide linkages, together with the possibility of re-utilising iodine after their deiodination. The presence of thyroglobulin-like molecules in the endostyle may well have favoured molecular orientation suitable for the polymerisation of the iodotyrosines.

But even with these advantages, thyroidal biosynthesis could hardly have become established unless the iodothyronines had some survival value, and it is difficult to suggest what this might initially have been. Indeed, it has been well said of the whole field of thyroid action that 'a huge and bewildering array of observations ... has generated more confusion than clarification'[17]. There are gaps, therefore, in the phylogenetic argument, and it is important to recognise them, for they are not peculiar to thyroid phylogeny. It has, however, been suggested that thyroidal biosynthesis might have served at first to sequester and bind iodine for general metabolic use[16], and that only later did the iodothyronines become involved in regulating growth, reproduction and metamorphosis, as they do in the lower vertebrates. The evolution of their calorigenic function in the higher forms was probably correlated with the establishment of homeothermy; responses of the gland to high temperature, which have been detected in lower vertebrates, may have provided the starting point. But these arguments remain a speculative contribution. They are part of 'the vastness of the unexplored realm' of endocrine phylogeny[16].

The steroid ring system

The iodothyronines are an example of molecules with latent hormonal potential, that had a wide distribution and long record before that potential came to fruition. Steroids, however, provide a much more ample illustration of this. Omnipresent throughout the biosphere, and with marked capacity for molecular diversification, they have been speculatively viewed as 'very ancient bioregulators' which evolved prior to the appearance of eukaryotes[45]. Their use as hormones is well established in two phylogenetically unrelated lines: the arthropods, where

ecdysteroids contribute to the regulation of moulting, and the vertebrates, where androgens and oestrogens regulate sexual characteristics and reproduction, while adrenocorticosteroids contribute to the maintenance of homeostasis.

The strong conservation of these vertebrate hormones (some of which are already present in agnathans) and of their biosynthetic pathways has led to the conclusion[45] that 'early hopes of establishing evolutionary tendencies' have not been realised, and that 'class divergences are due to environmental adaptation and not to evolution'. But some taxonomic diversification is discernible and, of course, environmental adaptation *is* evolution in action, revealing the power of selection to mould the individuals which are its targets[33]. The difficulty, as with the analysis of thyroid phylogeny, is that adaptive significance is not always easy to see.

One well-defined taxonomic feature is the presence of 1α-hydroxy-corticosterone in elasmobranch fish, where it probably acts as a mineralocorticoid. The existence in vertebrates of a 1α-hydroxylated derivative of vitamin D perhaps accounts for its origin[45]. Selective advantage can, however, be ascribed to the emergence of aldosterone as a mineralocorticoid in lungfish and tetrapods. Adaptation to terrestrial life may well have favoured a sharper separation of glucocorticoid action from mineralocorticoid, the requirement for this being a molecule sufficiently distinctive to be recognised by separate receptors. A further advantage of the separation could have been that it made possible some more precise action of the controlling mechanisms, with cortisol and corticosterone being regulated through the pituitary, and aldosterone mainly by the renin-angiotensin system[6].

Although these and other steroid molecules are highly characteristic of the vertebrate endocrine system, some of them occur widely in invertebrates and also in plants. Thus testosterone and progesterone are present in the haemolymph of the fleshfly, *Sarcophaga*[12], and testosterone in the serum and testes of the American lobster, *Homarus americanus*[8], while the in vitro conversion of cholesterol to vertebrate-type steroid molecules has been reported for the spiny lobster, *Panulirus japonica*[25]. Progesterone is among those reported in plants.

Whether these molecules have regulatory functions in invertebrates is still matter for speculation. As for plants, Heffmann[22], while accepting that the significance of the existence in them of vertebrate type steroids is unknown, has argued for the possibility that they may interact with plant chromosomes as they do with those of mammals. The evidence for this is, to say the least, tenuous, but it could well be that such molecules, both in plants and in invertebrates, have functions unrelated to chemical communication. Certainly it would be unwise to assume at this stage that they have no functions at all. As for the vertebrates, we can only draw the obvious conclusion that they have exploited the endocrine potentialities of widely distributed molecules, the functions of which in other groups remain obscure.

Peptide hormones

It is particularly in the field of peptide hormones that the most impressive advances have been made in our approach to endocrine phylogeny. Already they have amply confirmed, as was already apparent from thyroid and steroid phylogeny, that the boundaries traditionally set for the phyletic distribution of regulatory molecules have been far too narrow[43]. Developments in peptide chemistry have led to the precise characterisation of the molecules, while related techniques such as autoradiography, immunoassay, and immunocytochemistry have made possible the measurements of minute quantities of circulating hormones and the location of their sites of origin and of action. Such is the wealth and complexity of the continuously accumulating data that it will not be possible to do more here than indicate some emerging principles that bear upon phylogenetic issues.

One important advantage of peptide hormones, in the context of endocrine phylogeny, is that they give direct insight into the genetic mechanisms that generate adaptive diversification. Novelties can, in principle, arise at the level of the DNA code by point mutations bringing about amino acid replacements in peptide sequences, but the evolutionary possibilities of this are limited by the need to maintain the established properties of the molecules concerned. It is to be expected that strong selection pressure will conserve molecular structure, and thus ensure the stability of binding sites and other functionally important regions.

For example, insulin molecules from different species differ in respect of a number of substitutions, yet the agnathan (hagfish) insulin differs from that of man in only about 38% of its residues[35]. It is impressive to find such stability after some 500 million years of independent agnathan and gnathostome evolution.

Those substitutions that have occurred in the insulin molecule are predominantly conservative, which is why the variants share common biological properties[51]. Why, then, have these substitutions become established? Are they the result of random change, or are they adaptively significant? There is much to support the latter view, for neither internal nor external environments are static. Point mutations in biologically active molecules may therefore provide the fine-tuning needed to ensure their continuing efficiency in changing conditions[4]. Certainly in so complex a problem it would be unwise to assume that mutations with no immediately obvious function are necessarily non-adaptive. A more prudent view is that 'the concept that neutral mutations can account for a large proportion of sequence variations in proteins looks increasingly unattractive'[21]. It has also been argued that the individual is the ultimate target of selection; any neutral mutations that it may carry are therefore an irrelevant issue, and are 'merely hitch-hikers of successful genotypes'[33].

Interspecific variation is, of course, common in vertebrate peptide hormones, and has been reported also in the invertebrates. Thus extraction and characterisation of potent hyperglycaemic hormones from several crustaceans[32], including an isopod *(Porcellio)* and two decapods *(Carcinus* and *Orconectes)* have shown them to be peptides with 50–58 residues, with overall similarity of composition, but with much interspecific variation in detail. Cross-reaction studies have shown in this case that the receptors have varied side by side with the hormones. Despite the limitations restricting the evolutionary influ-

ence of point mutations in established molecules, some functional diversification is not precluded, particularly when associated with taxonomic separation sufficient to favour the establishment of new target relationships. This is well exemplified in invertebrates by the resemblance between the 8-residue erythrophore-concentrating hormone of crustaceans (ECH) and the 10-residue adipokinetic hormone (AKH) of insects. These two hormones, influencing respectively pigment cells and fat metabolism, are sufficiently alike structurally to imply divergence by point mutations from a single ancestral molecule[34]. With them can be grouped two further peptides, periplanetin CC-1 and periplanetin CC-2, which both show adipokinetic activity in grasshoppers and hyperglycaemic activity in cockroaches[4].

ECH Glu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH_2
AKH Glu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2
CC1 Glu-Val-Asn-Phe-Ser-Pro-Asn-Trp-NH_2
CC2 Glu-Leu-Thr-Phe-Thr-Ser-Asp-Trp-NH_2

Here, then, is a family of four peptides derivable in principle by point mutations from a common ancestral molecule, with some functional diversification.

Pathways of endocrine diversification

The evolutionary potentialities of point mutations become much greater when they are associated with gene duplication. Indications of repeated sequences (termed internal homologies) in large molecules (somatotropin is a case in point) suggest that they may have evolved through the association of the products of such duplication. But separation of the products has still greater potentiality, for this makes possible the continued functioning of the already established molecule while leaving the other product free to accept a range of mutations and thus to become available for new functions.

This is particularly well exemplified by two mammalian pituitary hormones: somatotropin (growth hormone), with 189–191 residues, and prolactin, with 199. Sequence studies have shown the ovine hormones to have some 23% of their residues in common, which is generally conceded to indicate divergence from a common molecular ancestry. A third member of the family, human placental lactogen, which shares some 85% of its residues with humor somatotropin, must have diverged much later, probably during primate evolution, with consequently much less time for mutations to accumulate[52]. The divergence of somatotropin and prolactin, however, must have occurred very early in vertebrate evolution, for both are present, on immunological evidence, in the agnathan (lamprey's) pituitary[53]. As a result, prolactin has had a long period of independent evolution during which it has established a range of target relationships so wide that it is difficult to attribute any one major function to it. In general, though, it seems to be particularly associated with transport-regulating effects[7].

It must suffice to mention one other example of gene duplication, with subsequent mutation, as a source of diversification. This concerns the dual series of neurohypophysial nonapeptides, represented in placental mammals by oxytocin

Cys-Tyr-Ile-Glu-Asn-Cys-Pro-Leu-Gly-(NH_2)
1 2 3 4 5 6 7 8 9

and either arginine vasopressin (Tyr[2], Phe[3], Arg[8]) or, in the pig and related forms, by lysine vasopressin (Tyr[2], Phe[3], Lys[8]). Because of the similarity of the two series, and because only one peptide (vasotocin: Tyr[2], Ile[3], Arg[8]) is found in agnathans, it is commonly assumed that the dual series arose through gene duplication during the emergence of gnathostomes[1]. Thereafter, some taxonomic diversification occurred by amino acid substitution in the several vertebrate classes, but this is not easy to correlate with selective pressure, except for the replacement of vasotocin by vasopressin at the origin of mammals, for the new molecule has the advantage of much greater antidiuretic activity.

Further evidence of gene duplication in this molecular family is found in the marsupials, some of which have been shown to possess two members of the vasopressin series[4]. Arginine and lysine vasopressin occur in two species of South American opossums, while lysine vasopressin and a previously unrecognised molecule, phenypressin (Phe[2], Phe[3], Arg[8]) occur in five Australian macropodid species. It can be assumed that gene duplication of the vasopressin gene occurred in marsupial ancestors, followed by independent mutation in the South American and Australian continents, but it is not yet possible to relate these postulated events to any form of selection pressure[9]. Further data, however, may be expected to clarify the problems presented by this remarkable phylogenetic series.

It remains to mention one other pathway for genetic diversification. This is post-translational processing and cleavage of large precursor molecules. A remarkable illustration of this is the production of a number of molecules, including several pituitary hormones, from proopiocortin[37], a single large (37,000 mol.wt) polypeptide. In the corticotroph cells of the mammalian pituitary it yields corticotropin and β-LPH (β-lipotropin) as final products, but in the melanotroph cells the corticotropin is further cleaved to yield α-MSH (melanocyte-stimulating hormone) and CLIP (corticotropin-like intermediate lobe peptide), while β-LPH yields β-MSH, β-endorphin and another fragment. This sequence of events seems to have been established very early in vertebrate phylogeny, for the precursor is present in the salmon, but with some distinctive characteristics[26]. It is thought also to be present in agnathans, although it may there be appreciably different from that of gnathostomes[38]. The origin of the precursor is unknown, as also is the history of its products, but the presence in it of repeated MSH-like sequences suggests that, as with somatotropin, gene duplication, with association of the products, may have played some part.

Leaving aside many matters of detail which are outside the scope of this account, one cannot fail to be impressed with the remarkable flexibility of the genetic mechanism in providing a basis for the emergence of novelty and the establishment of major adaptation, while also ensuring molecular stability and any fine-tuning needed for functional efficiency. Acher[1] emphasises the complexity of these processes, and the many places at which variations can occur. The peptide molecule must be produced in a stable form adequate to fit its receptors, while the confor-

mation of the latter is itself the result of evolution. Add to this the specialisation of the target cell, and one can appreciate the complex chains of intermediate molecules and enzymes which have to be integrated to secure adaptively valuable results. Natural selection is the only known mechanism which can integrate so many initially disparate factors into an orderly phylogenetic sequence.

Multiple sites and actions

New insights into the increasingly complex area of endocrine phylogeny have been provided by evidence, primarily but not entirely immunocytochemical, that many known peptide hormones of vertebrates may also be present in identical or closely related forms in the nervous system, and particularly in the brain[1]. The alimentary hormone cholecystokinin (CCK) is one example, with 33 residues in the alimentary mucosa, where it was first identified, but mainly with 8 in the nervous system. The difference may be attributed to differential processing of a macromolecular precursor. Conversely, somatostatin, first identified as a factor transmitted in the hypophysial portal system to inhibit the release of somatotropin, was later identified in alimentary sites, notably in the D cells of pancreatic islet tissue, where it is thought to inhibit the release of insulin from the B (insulin-secreting) cells by local (paracrine) action[51]. It must be remembered that antigenic determinants may comprise only a few amino acids, and that the sequence involved may not include that part of the molecule responsible for biological activity. Nevertheless, the evidence is already sufficiently powerful to justify current viewing of the nervous system as a secretory centre of great complexity.

With the wisdom of hindsight, one can see this as a corollary of the existence of neurosecretory cells. These, according to an earlier operational definition[48], were distinguished from conventional neurons because they discharged chemical mediators (neurohormones) that were conveyed in the blood stream to function at a distance like the hormones produced by epithelial endocrine glands. These neurohormones were later shown to be peptides; the cells secreting them were therefore termed peptidergic, distinguishable from the cholinergic or aminergic conventional neurons. However, the great variety of chemical signals now known to be released in the vertebrate central nervous system has weakened this distinction. It has been suggested instead[24] that two types of transmission might be recognised: 'anatomically addressed' ones, involving point-to-point transmission in the central nervous system, and mediated principally by γ-aminobutyric acid and L-glutamate, and 'chemically addressed' ones, dependent upon the monoamines and the many neuropeptides, of which over 30 have now been identified. It is proposed that 'chemically addressed' systems are characterised by a rich diversity of signals, a slower time course, and less precise connections than are provided by the 'anatomically addressed' ones[24]. Be this as it may, the brain is certainly emerging as a vastly complicated neuroendocrine secretory organ. Associated with this view is recognition that neural signalling and endocrine regulation are not two distinct activities with independent origins, but rather as parts of a regulatory complex from which the endocrine system sensu stricto separated and then followed its own course. To examine the fuller implications of this approach it will be necessary briefly to consider the situation in invertebrates.

Invertebrates

That invertebrates are likely to have much in common with the vertebrates in these respects is suggested by immunocytochemical studies of protochordates which, as we have seen, take us back close to the remote ancestors of vertebrates. Positive results have been obtained for a wide range of vertebrate-like peptide material in the alimentary tract and cerebral ganglia of the ascidian *Ciona*[18]. Cells in the alimentary epithelium of *Branchiostoma* (amphioxus) react to antisera against a number of mammalian peptides, including insulin, glucagon, pancreatic polypeptide, somatostatin, secretin, vasoactive intestinal polypeptide, pentagastrin and neurotensin, and show specific distribution patterns[42]. Further, the brain has neurosecretory systems of surprising complexity for so small an animal. Monoaminergic and peptidergic neurons have been identified, while a central neurohaemal area is thought to be comparable to the median eminence and neurohypophysis of vertebrates[39,40].

Amongst other (non-chordate) invertebrates it is the insects that have been most closely studied, and here, although conclusive demonstration of the neurotransmitter roles of suspected agents are still needed, there is ample evidence that many vertebrate neurotransmitters are also active in these animals and in other invertebrates as well[23]. Substances thought to act as neurotransmitters in insects include acetylcholine (predominant in the central nervous system) and glutamic acid (at excitatory neuromuscular junctions), with monoamines acting at various sites. Neurosecretory peptides are present throughout the central nervous system of these animals (bursicon and eclosion hormones being examples characteristic of the group), but there is also immunological evidence of a range of substances resembling such typical vertebrate products as somatostatin, insulin, glucagon and gastrin.

Comparisons with vertebrates need, however, to be drawn with caution. For example, immunoreactive somatostatin has been found in two species of pond snail (*Lymnaea stagnalis* and *Physa* spp.) and has been thought to be a growth factor[19], but it seems not to be chemically identical to synthetic somatostatin. Again, the ganglia of the mollusc *Aplysia* and of two slugs contain a neurohypophysial peptide-like material which resembles vasotocin and vasopressin, but is actually neither. It is suggested[46], although quite hypothetically, that this material might act as a neurotransmitter or neurohormone in the regulation of fluid balance.

These data, and much else besides, are confirming the view that the phylogenetic history of vertebrate hormones must extend outside the group. The strong indications of insulin-like material in a number of invertebrates provide a good illustration of this, studies of insects being particularly convincing. Extracts of the brain of the blowfly *(Calliphora)* contain a substance similar to insulin in physicochemical properties as well as in biological activities[14], while insulin B-chain immunoreactivity has

been located in a few cells in the frontal ganglion of the tobacco hornworm *(Manduca)*[15]. A complication still awaiting interpretation in terms of molecular evolution, however, is the demonstration in the adult silkworm moth *(Bombyx)* of three forms of a well-defined insect hormone (prothoracotropic hormone) and of a significant resemblance between these and the A-chain of insulin[36].

The possible functions of insulin-like materials in invertebrates remain to be discovered. They have been thought to be involved in the regulation of carbohydrate metabolism in bivalve molluscs[41], but probably to have no glucostatic role in the lobster *Homarus americanus*[44]. Of course, the functions of endocrine molecules need not always be the same, regardless of the group in which they occur. A case in point is the detection in the central nervous system and haemolymph of the pond snail *(Lymnaea stagnalis)* of an immunological response resembling that of the thyrotropin-releasing hormone (TRH) of the vertebrate pituitary. Obviously the action of this substance, whatever it may be, cannot be that of the vertebrate hormone, which is transmitted in the hypophysial portal system to evoke release of thyrotropin. There is, however, experimental evidence that exogenous TRH may influence hydromineral balance in gastropod molluscs by an action on the secretion of sulphated polysaccharides by the epithelium of the foot[20]. Too little attention is sometimes given to the profound differences in organisation and mode of life when comparisons are made between widely separated taxa[3], added to which there is a tendency (understandable in the absence of any other clue) to look for familiar functions in preference to exploring novel paths. There are problems here, both intellectual and methodological, which are fundamental to phylogenetic analysis.

Insulin, it should be added, is only one amongst many immunoreactive materials resembling vertebrate-type peptides which have been identified in invertebrates, but one other example must serve. The ascidian (protochordate) *Styela clava* is believed, on immunological and experimental evidence, to secrete a peptide with CCK-like properties, associated with a receptor system more generalised than that of the vertebrate alimentary tract, but capable of recognising vertebrate peptides[50].

Nor is such evidence confined to the chordate line. CCK is not easily distinguishable immunologically from gastrin, another alimentary hormone with which it shares the same pentapeptide sequence, but CCK/gastrin-like material has been demonstrated immunocytochemically in invertebrates as diverse as the ectoproctan *Bugula*, an earthworm, hydra, and an anthozoan[29]. But the evidence of wide distribution of certain peptides extends also to unicellular organisms and prokaryotes. Radioimmunoassay and bioassay have identified insulin-like material in the protozoan *Tetrahymena* and in the prokaryote *Escherichia coli*, as well as in two fungi *(Neurospora* and *Aspergillus)*, while corticotropin-like and somatostatin-like immunoreactions have also been found in *Tetrahymena*[30]. It remains to be shown whether or not such materials function in any form of chemical communication, but evidence already suggests that receptors and effector pathways which would be expected to be associated with them are also present[43].

Challenging perspectives

This line of thought implies that the fundamental biochemistry of chemical communication must be very ancient indeed, and that phylogenetic advances have been founded upon an initially limited range of messenger molecules. The initial selection of these would have demanded a capacity to associate with receptors which can be visualised as having perhaps evolved out of protein subunits already present in cell membranes, and capable of assembly into appropriate configurations[11]. There is evidence that such associations already exist in unicellular organisms such as *Tetrahymena*. Phylogenetic exploitation of these associations and of their constituent molecules in multicellular forms would have involved, as already suggested, the functional diversification of endocrine and nervous systems out of a common ancestral complex[43], in which pluripotent cells of epithelial origin provided for external relations as well as for internal regulation[48]. Of course, there is still much that is speculative in these arguments. But they are opening up challenging perspectives, and they demand testing through a much wider range of observations and experiments, drawing, let us hope, upon many more species than the few that have so far been used.

* We regret the untimely death of Prof. E. J. W. Barrington in December 1985.

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