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DARWIN REVIEW

A Darwinian view of metabolism: molecular properties determine fitness

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Abstract

'Nothing in biology makes sense except in the light of evolution'
Theodosius Dobzhansky

Why do organisms make the types of chemicals that they do? Evolutionary theory tells us that individuals within populations will be subject to mutation and that some of those mutations will be enzyme variants that make new chemicals. A mutant making a novel chemical for that species will only survive in the population if the 'cost' of making the new chemical is outweighed by the benefits that result from making that molecule. The benefits, or adverse consequences, that a novel chemical X can confer to the individual organism are not a property of the simple existence of X in the cell but can be traced to one of the multiple properties that X will possess because of its molecular structure. By considering only three basic types of molecular property and by considering how selection pressures will differ for each kind of property, it is possible to account for much of the chemical diversity made by organisms. Such an evolutionary model can also explain why the properties of enzymes will differ depending on the molecular properties of the chemicals they make, and why the widely accepted terms 'primary metabolism' and 'secondary metabolism' have been so misleading and unsatisfactory.

Key words: Evolution, natural products, primary metabolism, secondary metabolism.

Why did the theory of evolution have so little impact on biochemistry?

Until Darwin and Wallace conceived the general principles of evolution, studies of the structure and function of organisms and their classification lacked a clear context. The principles of trait variation, its inheritance, and natural and sexual selection on variants via differential survival and reproduction (i.e. fitness) explained why related organisms might differ in important respects and why unrelated organisms might also share common, ancestral features. Biological diversity was no longer something to be described and classified, but something to be understood and explained in terms of simple, general rules.

Biological diversity is underpinned by chemical diversity, consequently the principles of evolution must apply to chemical diversity[1] in organisms. However, while evolutionary theory has dominated thinking in the biological sciences, it has been much less influential in biochemistry. This could largely be a consequence of the fact that biochemistry did not grow from biology but from chemistry. Until the early 20th century, biochemistry was called 'physiological chemistry', and that subject was studied in chemistry departments. In the last quarter of the 19th century, physiological chemists had colleagues who would be more excited by the Mendeleyev Table and emerging ideas about atomic structure than they would about the evolutionary ideas of Darwin or Wallace. To make matters

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[1] In this review we shall only be considering the chemical diversity that exists in an organism that results from that organism's own metabolic processes. This metabolic chemical diversity excludes molecules that are actively or passively taken up but not subjected to significant metabolic transformation.

worse, in 1891 the German physiological chemist Albrecht Kössel (who won the 1910 Nobel Prize for Medicine) unknowingly initiated a schism when he proposed that plants had two distinct types of metabolism, 'primary' and 'secondary'. Kössel proposed that 'primary metabolites' were involved in basic processes of the cell and common to all organisms, while 'secondary metabolites' were made by distinct pathways limited to only a few organisms; these chemicals therefore being of less general importance. The physiological chemists starting the new biochemistry departments were almost exclusively those studying Kössel's primary metabolism, while the study of secondary metabolites was relegated to those left behind in chemistry departments[2]. So the scientists who were most doggedly exploring and cataloguing the chemical diversity of the natural world, and who were the people most likely to gain insights from evolutionary theory, were to spend the next century discovering tens of thousands of new natural chemicals blithley untroubled by the question as to why organisms were making these compounds. Meanwhile, the discipline of biochemistry became a subject dominated[3] by animal studies (especially mammals) which conveniently generally lacked 'secondary metabolism'.

By the end of the 20th century, a new strand of research, 'molecular biology', forced evolution back onto the agenda of biochemists. The rapidly growing databases of DNA sequences from different species and the algorhythmic tools that allowed inter- and intra-species comparisions, made it possible to trace the lineage of specific enzymes. Discussions of the evolution of enzymes capable of specific chemical tranformations became commonplace and were seen as a useful way of linking amino acid sequence information to the 3-D structure of the proteins and, consequently, to the specific functional features (e.g. binding sites) of particular enzymes. Nevertheless, despite interest in the evolution of enzymes, little attention has been paid to the larger picture of the evolution of metabolism as a whole (Jensen,1976; Chapman and Ragan,1980). Even when a broader view has been attempted, the focus has tended to be on one branch of metabolism. In animal biochemistry, for example, the evolution of 'primary metabolism' has been considered for organisms living in extreme environments (Hochachka and Somero, 1984). In plants, the selection pressures that have shaped photosynthetic metabolism have also been addressed; perhaps an inevitable consequence of the discovery of more than one enzyme capable of fixing carbon dioxide. In microbes, selection forces operating on microbes in restricted growth media have also been well explored. However, what general rules have been found to explain the evolution of metabolism, rather than that of enzymes, in these studies? Rather few. This became apparent when we initiated a debate (Jones and Firn, 1991) about the evolution of 'secondary metabolites', the largest and most economically important chemical diversity on the planet. After we had developed some basic 'rules', based on the conjunction of physicochemical and evolutionary principles that we proposed would govern selection of 'secondary metabolites' (or Natural Products as most chemists still call such substances), we increasingly realised that these 'rules' must be a subset of more general rules that govern the evolution of any type of metabolism. However, in discussions and debates about our evolutionary model to explain the diversity of Natural Products we frequently found ourselves tripping over the terms 'primary metabolism' and 'secondary metabolism'. In particular, we were troubled by the fact that many very important chemicals (e.g. lipids, carotenoids, polysaccharides) made by organisms do not adequately fit into either of these two categories! In this short review we outline an evolutionary framework that can be applied to all metabolism and we shall argue that the terms 'primary metabolism' and 'secondary metabolism' are not helpful and have no useful evolutionary basis. We will consider one central aspect of the evolution of metabolism: what rules determine whether a new biosynthetic capacity arising through mutation is retained? By thinking about the way in which selection might have shaped metabolism, insights can be gained about the properties of enzymes and the roles of the chemicals that these enzymes make.

What determines the fitness of a mutant making a new chemical?

We start by considering, in very general terms, the selective consequences of a new biosynthetic capacity in an individual that produces only one new chemical as a result of a mutation[4]. In this case, it is the *intrinsic* properties possessed by that new substance, not the properties of the mutated enzyme, that will be the initial focus of selection. The new substance could:

(i) possess properties that are new and enhance the functioning of the cell and hence the organism

(ii) possess properties that are new and adversely affect the cell and hence the organism

(iii) possess properties that are new but have no impact on the functioning of the cell or the organism other than the imposed metabolic cost of production

(iv) possess properties that can substitute for an existing, necessary property with no impact on the function-

[2] The chemists were, not surprisingly, unimpressed by Kössel's use of the term 'secondary' for the kind of chemicals made by organisms that had been bequeathed to them by the biochemists so chemists continued, and still continue, to use their old term 'Natural Products' for this class of compounds.

[3] An analysis of the general biochemistry textbooks available in the University of York library in 2000 showed that the terms 'secondary metabolism', 'secondary metabolites', 'Natural Products' were very rarely found in any index. In effect, the majority of the natural world's biochemistry was missing from the syllabus!

[4] It is generally believed that the evolution of metabolism is highly, but not absolutely, dependent on gene duplication so that a mutation in one copy of a gene can lead to a new product while the original pathways of metabolism are maintained by another copy of the original gene.

ing of the cell or the organism other than the imposed metabolic cost of production, but with the accrual of potential functional redundancy

(v) possess properties that can substitute for an existing, necessary property with a negative impact on the functioning of the cell hence the organism (via, for example, diversion of substrates)

If the new molecule possesses *intrinsic* properties giving an organismal cost/benefit <1 then selection will favour the retention of individuals possessing that variant relative to those that do not (Fig. 1). Variants with a cost/benefit >1 will be lost from the population at a rate depending on the degree to which the cost/benefit ratio exceeds 1. However, what happens if the new substance is converted into a second substance by an existing enzyme? Now selection can act on the *intrinsic* property of the original new substance and/or the *intrinsic* properties of second new metabolite(s). Thus when a new substance feeds into an existing metabolic matrix, the focus of selection could be on the properties of one or more *derived* compounds (Fig. 1).

What molecular properties are subject to selection?

Having placed the emphasis of evolution of metabolism on the properties of chemicals being made, rather than enzymes making them, it is necessary to consider the types of physicochemical properties that substances possess that could act as a focus for selection. Like human characters, chemical characters have many facets to their personality and more than one facet may play a part in selection. However, there seems to be a few dominant physicochemical characteristics which are summarized below.

Biomolecular activity: pathways leading to physiologically active compounds: Because of the nature of protein/ligand interactions, it is necessary for a ligand to have a structure that precisely fits the binding site on the protein with which it interacts if those binding sites are to be significantly occupied when the ligand is present at physiologically realistic concentrations[5]. These are very strict constraints and therefore very few chemicals arising via mutation will possess the appropriate structure to bind to a protein when both the ligand and the protein are present at low concentrations. We have defined the ability of a chemical to interact with a protein at low concentrations, such that the function of the protein is affected, as 'biomolecular activity', to distinguish it from the less well-defined 'biological activity' (Firn and Jones, 2000). The low probability of any chemical possessing potent biomolecular activity must have been a severe evolutionary constraint on the ability of an organism to have enhanced fitness by producing chemicals with potent biomolecular activity. Consequently it was proposed (Jones and Firn, 1991; Firn and Jones, 1996) that the capacity to generate new chemical diversity would have been a general trait that was selected for in organisms making such chemicals. A large chemical diversity begets a high probability that a mutant can make a compound with beneficial biomolecular activity. It was also proposed that metabolic traits that fostered the retention of existing chemical diversity, even in the absence of a current role for some products, would have also been selected for, provided the current cost of production was outweighed by the current benefits. Some of the predicted traits, such as a relaxed substrate specificity, were considered controversial when first proposed because they were at odds with the view that enzymes always had high specificity. However, the view (sadly still widely taught at elementary level) that enzymes are always highly specific was a consequence of the fact that most biochemists working on enzymes in the 20th century were mainly studying enzymes involved in 'primary metabolism', where such high specificity had been favoured by selection (see further in this article). Enzyme specificity should be seen as a relative term and it should be recognized that high specificity is the result of intense selection and not an inherent property of enzymes.

An operative enzyme variant might only possess new enzyme activity because of a loss of substrate specificity, and the rate at which the new variant mutates further to increase the specificity for a new substrate will be very dependent on the selective advantage that results from increased specificity. For example, if there is no other substrate in the cell (at concentrations that are metabolically significant) then the selection pressure for increased substrate specificity will be negligible. Even if an alternative substrate is available and is being converted at a significant rate to another product but that substance has properties that are selectively neutral, there might be no selection pressure favouring increased enzyme specificity. In other words it is no longer realistic to consider enzymes as being substrate specific, rather, some enzymes, as a result of selection, possess high substrate specificity.

Specific physiochemical properties: pathways leading to chemicals with a beneficial physicochemical property: When chemists began to isolate and characterize the chemicals found in organisms, they often grouped chemicals sharing similar physicochemical properties into broad groups: pectins, hemicelluloses, polysaccharides, lipids, carotenoids, flavonoids, phenols, and the like. The shared physiochemical properties of a group enabled them to be extracted or quantified together. For all such broad classes of naturally occurring molecules, a considerable diversity of individual chemical types was found within the group. Why does one organism make such a diversity of lipids, carotenoids or polysaccharides? Why does one bacterial species make a different spectrum of lipids compared to another species? These groups of substances are the ones that have been so awkward to fit into the categorization of 'primary metabolite' or 'secondary metabolite'. For

[5] The Law of Mass Action dictates the relationship between the concentration of a substance in solution and the proportion of target binding sites occupied. The better the fit between ligand and binding site, the lower the concentration of the ligand needed to fill 50% of the potential binding sites.

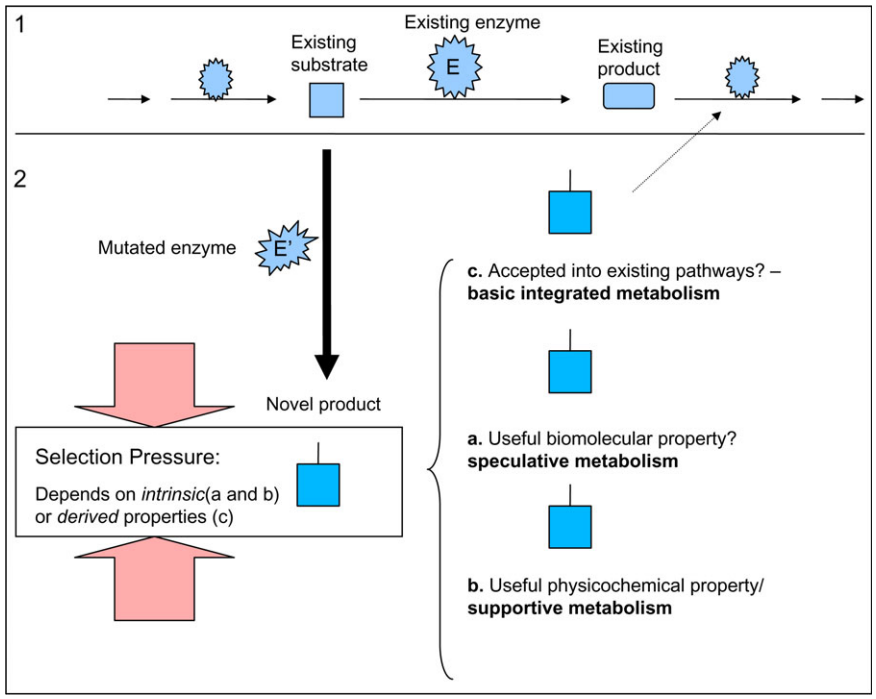

Fig. 1. When a novel substance is produced from an existing substrate as a result of a novel enzyme activity arising by mutation, at least three different properties of the novel substance will be subject to independent selection. Because the selection forces for each property class differs, it is predictable that there will be significant differences between the metabolic traits contributing to each property class.

example, considered as a group, lipids would seem to be 'primary metabolites' in that they are essential for the short-term functioning of the cell and all cells contain a mix of lipids. But if one considers some individual lipids, they would seem more like 'secondary metabolites' in that they are often made only by a few species, and when their synthesis is inhibited the cell can suffer no short-term disadvantage (see later references). How can one resolve this apparent paradox, where lipids as a group seem to be 'primary metabolites' but some individual lipids fit the description of 'secondary metabolites'?

The answer is that it is a broad physicochemical property that is being selected for in such molecules. Because that property is not strictly linked to the detailed fine structure of the molecule a wide tolerance for structural variation exists: many similar structures have some common physiochemical properties[6]. Consequently when a chemical variant arises within one of these broad classes, there is a high probability that the new substance will possess similar physiochemical properties to that of the product made by the original enzyme (this is in stark contrast to the very low probability of the chemicals possessing equivalent biomolecular properties). If the new and the old products share similar physicochemical properties, then there is a reasonable chance that some such mutations will be selectively neutral. It is therefore predictable that if certain types of physiochemical properties are useful to cells, but the properties are not highly structure-specific, then a diversity of chemical types will be found within a single species. Thus some of the diversity of carotenoids in an individual species can be explained: there are simply no sufficiently strong selection pressures operating either to limit diversification or to optimize a single structure to serve this function. It is also predictable that once a diversity of molecular structures begin to provide a way of endowing the cell with an effective physicochemical capacity (e.g. to form a functioning membrane), then different organisms will achieve similar functional optima using different proportions of rather similar molecules. This would explain why individual species might possess distinctive mixes of any of these substances. The diversity of flavonoids, xanthophylls or lipids that characterize so many species is simply an inevitable and predictable consequence of the fact that cells need some chemicals with certain properties, but when those properties do not involve any specific protein–ligand interactions then a considerable tolerance exists and 'good enough' is sufficient to retain the mixture in the population[7].

There can also be an advantage to an individual organism in producing chemical diversity if the chemicals being made contribute to the physical defences of the producer by

[6] The fact that all organisms possess groups of chemicals with similar physicochemical properties allowed the early chemists to use the gross common properties to 'fractionate' extracts to produce mixes of chemicals that shared this dominant common property: lipids, alkaloids, pectins, etc.

[7] For those who like sporting analogies, 'biomolecular activity' is akin to singles tennis or golf where one person can get to the top while 'physicochemical properties' are akin to team sports where a team can win, even when individual substitutions have been made.

helping to exclude other organisms from the cell or organism (e.g. the cell wall, the cuticle). For example, micro-organisms seeking to invade plant cells have to degrade the cell wall, and a chemically diverse cell wall would be expected to be less susceptible to degradation than a chemically homogeneous one. The cuticle offers some protection against microbial invasion, hence a chemical diversity of cuticular waxes is predictable.

The prediction that chemical diversity will be tolerated in compounds serving a largely physicochemical role is supported by a number of recent studies of *Arabidopsis* plants. Certain carotenoids can be absent without apparent effect (Pogson *et al.*, 1996). Some individual fatty acids are not essential for growth, development, and photosynthesis (McConn and Browse, 1996). Changing the cuticular waxes need not have an adverse effect (Jenks *et al.*, 1995). Clearly such inherent redundancy is not absolute and these studies only provide evidence of substitutability under the specific conditions employed by the investigator. However, it is evident that the significant substitutability within these classes of compounds can be found and is predicted on the basis of their shared physicochemical properties.

The chemical diversity found in groups of chemicals retained for their physicochemical properties may also be a valuable resource as a pool of chemical diversity to be drawn upon via mutation for the generation of new physiologically active compounds (i.e. biomolecular activity). Furthermore, the fact that some enzymes involved in making physicochemically useful substances might have low substrate specificity gives an organism a reportoire of enzymes that may promiscuously produce substances with biomolecular activity. There is evidence consistent with this prediction. The internal cell regulators, IP3 and diacylglycerol, are derived from a lipid (Exton, 1994) as are the prostaglandins (Smith *et al.*, 1991) and jasmonates (Vick and Zimmerman, 1984). The carotenoid pathway serves to provide the precursor of abscisic acid (Zeevaart *et al.*, 1991) and the fungal mating substance trisporic acid (Bu'Lock, 1973). There are numerous examples of small molecules derived from cell walls possessing biological activity which may be important in plant–microbe interactions (Boller, 1995). These examples illustrate the important point that some pathways are making substances which serve rather different roles (physicochemical and biomolecular, for example) hence one cannot assign functions to substances on the basis of their route of synthesis.

When one considers how selection might operate on pathways which participate in the production of substances with useful physicochemical properties, it would seem that the forces of selection would not be that dissimilar from those operating on pathways that have evolved to increase and retain biomolecular diversity. Consider an enzyme currently playing a role in the synthesis of an orange pigment. If a mutation causes that enzyme to become less substrate-specific such that it now acts on another substrate as well as the original one, but both the new product and the original product possess molecular structures that cause them to absorb wavelengths in the orange/yellow part of the visible spectrum, then the mutant may experience no loss of fitness and there will be no selection pressure for the original, narrower substrate tolerance. Note in the scenario just given, there is no need for gene duplication to occur at some stage prior to the mutation of one copy of the gene because the function of the original gene is adequately retained in the newly mutated gene. Interestingly, a number of the predicted metabolic traits originally proposed for pathways contributing to the generation and retention of metabolic diversity now appear to be shared with pathways contributing chemicals with useful physiochemical properties to cells.

Derived properties: integrated pathways involving the basic metabolic pathways of most cells: The two properties discussed so far, biomolecular activity and physicochemical properties, provide a rationale for selection that can explain much of the chemical diversity of the natural world. It is now necessary to explain the chemistry that is largely shared between organisms, what is traditionally called 'primary metabolism'. Unlike the previous types of metabolism where selection has not ruthlessly reduced molecular diversity, 'primary metabolism' is characterized by a remarkable lack of metabolic diversity between organisms. This fact provides the clue to the molecular properties that have shaped 'primary metabolism'. In contrast to selection of biomolecular activity and physicochemical properties, we propose that the key feature in the evolution of 'primary metabolism' is that selection does not act on the *intrinsic* properties of new molecules that arise through mutation. Indeed the two kinds of *intrinsic* molecular properties discussed so far (biomolecular activity, useful physicochemical property) are usually absent[8] in 'primary metabolites'. Instead, selection of 'primary metabolism' is based on the capacity of any new molecule that is made being compatible with the existing pathways that lead from that point. It is an extreme example of the property being selected for being *derived* rather than *inherent*. In other words, once an operational metabolic pathway evolves to feed useful molecules up a metabolic chain, selection operating on the individual enzymes in that pathway are, in effect, reducing the opportunity to accept different molecular structures into that pathway at some future time. The collective properties of that small branch of metabolism begin to dominate selection rather than the individual properties of any new molecule that arises via mutation (i.e. cannelization). Furthermore, because each small branch of 'primary metabolism' usually links to other branches in a network, the properties of the network will soon dominate the selection in the individual branch of metabolism. Indeed one could argue that the term 'primary metabolism' would be better replaced by a term such as an 'integrated metabolic network'.

[8] Few 'primary metabolites' are highly pigmented; 'primary metabolites' share no common physicochemical properties with each other (or the other major classes of physicochemical useful substances); and few possess biomolecular activity akin to that found among a few members of the isoprenoid, alkaloid or phenylpropanoid families.

Interestingly, this view that 'primary metabolism' (an integrated metabolic network) is driven by molecules serving a highly derived role in the service of the whole network, meshes quite well with the two previous ideas about the evolution of 'primary metabolism'. Horowitz (1945) postulated that biochemical pathways leading to the building blocks necessary for the production of structural and informational molecules (RNA, DNA) evolved 'backwards'. New enzyme variants that could introduce appropriate molecules into the evolving pathway would be highly beneficial and would be strongly selected for. This would be an extreme example of what we define as selection of a *derived* trait, in that each new variant contributes to fitness by improving the efficiency of production of a substance that already possesses a useful property. An alternative model, where diverse and random biochemical transformations generate a co-ordinated function by chance (Kaufman, 1993) is an even more extreme version of a property being *derived*: in this model the *derived* property resides within the unique collective properties of all the components. The important features shared by both these models is that 'primary metabolism' would first have evolved because chemical diversity was available and was then extended by chance events. Once a self-replicating structure evolved, the main biochemical processes involved in the production of that structure would be severely constrained. A new enzyme variant arising which could produce a new molecule from a common, important precursor in a cell would be likely to have adverse consequences to the organism simply as a result of disrupting the flux of material through that pathway. Although gene duplication can permit extension or rather substitution of chemistries, competition for substrates would have existed and would usually be highly detrimental. The new product might also have sufficient structural similarity to an existing metabolite that it might act as a substrate analogue for another enzyme or act as an allosteric inhibitor, both of which might have had adverse effects on the cell. These types of constraints will have been very severe for all pathways through which there is a high metabolic flux and which are necessary for cell homeostasis. Because the selection pressures operating on this type of pathway are so different from those operating on pathways leading to molecules selected on the basis of their intrinsic properties, it is predictable that metabolic traits will differ. For example, high substrate specificity is predictable in enzymes participating in this type of metabolism because selection would favour enzymes that carry out one role very precisely. The fact that 'primary metabolism' has been unchanged for billions of years means that there has been ample evolutionary time to allow each and every enzyme involved in this type of metabolism to be tuned by selection for group compatibility.

'Primary' and 'secondary' metabolism are now outmoded terms

By thinking in terms of the properties of molecules rather than chemical structures one can predict that every organism should possess a collection of molecules with appropriate properties; yet it is the properties that are needed, not specific molecules. Consequently, one expects that evolution might cause structural radiation within a functional category such that different molecules might play similar roles in different organisms because they share similar properties (chitin versus cellulose; starch versus inulin; etc.). Likewise, similar chemicals may play different roles in organisms because they can possess more than one property (flavonoids acting as UV screens, as signalling molecules, and as defences). The recognition that it is the properties of individual molecules that determine their value as a part of metabolism, and that a pathway can contribute to different property classes, reveals the inadequacy of the classification of metabolism into 'primary' and 'secondary' (Mann, 1987). This classification is usually ascribed to Kössel (Kossel, 1891; Mothes, 1960) but Hartmann (2007) has recently argued that, some decades before Kössel made his proposal, the great German plant physiologist Julius Sachs used two different terms (translated into English as 'inner economy' and 'by-products' to describe the same two classes. However, both nomenclatures really did little more than note that all living organisms seem to share many chemicals while placing the remaining chemicals into nothing more than a 'miscellaneous' category. Neither Kössel nor Sachs attempted to seek underlying principles on which to base their categorizations. Remarkably, Kössel's terms are still used, despite that fact that his classification system fails so dramatically when applied to so many important metabolites. Some major, functionally important chemical groups (lipids, carotenoids, polysaccharides, cuticular waxes, etc.) are still sometimes classified as 'secondary' despite the fact that all organisms require lipids and mutations that eliminate carotenoid synthesis in plants are usually lethal. Is gibberellin A_1 secondary because it is made via the isoprenoid pathway or primary because it plays a role as an endogenous regulator? Many inadequate classification systems have survived in biology for long periods of time, but usually because they offered some useful insight or were operationally convenient. But in what way has Kössel's division of metabolism into 'primary' and 'secondary' helped generate insights into metabolism or were operationally convenient[9]? Perhaps the prolonged use of such a poor classification system owes more to the fact that Kössel gave the term 'primary' to the type of metabolism that had been taken up for study by the new biochemistry departments and justified leaving behind the secondary metabolites (or Natural Products) for chemists to study. We would argue that it is time to leave behind Kössel's classification because it lacks an evolutionary basis, it provides no productive insights about metabolism, and does not adequately classify many molecules made by organisms, and thus inhibits

[9] Although Kossel's terminology is often used today, they were not widely used by his contemporaries, especially those working on plants. For example, Julius Sach's highly influential textbook categorised metabolites simply into those commonly needed to make cells and 'the by-products' (Hartmann, 2008).

progress toward a unified understanding of the evolution of metabolism.

Basic integrated metabolism.

We are proposing that the pathways of 'primary metabolism' are simply pathways where there has been a very strong and ancient canalization. In such pathways, at an early stage in evolution, selection operated on the individual enzymes to ensure the proper functioning both with respect to the adjacent elements of the pathway and also with the overall metabolic network sustaining the cell. Over evolutionary time the integration of the individual pathways became tighter, and it became harder for selection to improve the overall functioning of the network by selectively improving the functioning of any one element within it. Pathways where the majority of substances being made are selected on the basis of their derived properties—properties which serve the needs of the overall metabolic network—might well be called parts of a 'basic integrated metabolism'.

Supporting metabolism

In contrast to 'basic integrated metabolism', the metabolism giving rise to molecules with intrinsic or derived physicochemical properties will be much less highly integrated. This is a consequence of the fact that the substances being made are highly substitutable as a result of the fact that the properties that give each value are shared by a range of structurally related substances. This type of metabolism largely serves to generate substances that contribute vitally to the physical environment in which 'basic integrated metabolism' functions. The lipids in membranes, the pigments in membranes, the light-screening pigments in vacuoles, and the components of cell walls would be typical members of this diverse group.

Speculative metabolism

Because specific, potent biomolecular activity against a defined target protein is such a rare property for a molecule to possess, organisms that gain fitness by making such substances must have faced a severe evolutionary challenge (Jones and Firn, 1991). The pathways that contribute to such metabolism are selected on the basis of their capacity to make, at low cost, the rare, sometimes ephemeral property of biomolecular activity. The selection pressures will be different, or applied to a different degree, from those operating on 'basic integrated metabolism' or 'supporting metabolism'. Furthermore, in the case of selection on pathways leading to molecules with biomolecular activity, the forces of selection will change more rapidly than the forces of selection operating on the other forms of metabolism. The homeostatic capacity of the whole organism reduces the selection pressures that are operating on 'basic integrated metabolism' or 'supporting metabolism' so the rate of change in such metabolism is predictably less than in the metabolism giving rise to molecules with very specific intrinsic properties. For example, a new pest or disease can very rapidly (less than one generation) place a massive selection pressure on pathways leading to biomolecular activity (and perhaps on pathways leading to certain kinds of physicochemical properties such as resistance to microbial penetration). However, it is also predictable that the initial stages of a biochemical pathway evolved on the basis of generating compounds with intrinsic properties might gradually become one that has some properties of a pathway selected on the basis of derived properties. It is also predictable that the evolution of a molecule with biomolecular activity which acts in the producing organism itself or on the same species (hormone or pheromone), generates a quite different selection pressure to the production of a chemical that acts on another species. Thus there can be no firm boundaries between each of the three broad classes proposed because, after every mutation giving rise to new chemical in an organism, selection will take place by applying all the selection rules from each of the three classes. Finally, there may be other property classes that remain to be identified that will add additional rules.

Summary

These complexities suggest to us that it is time to begin to discussing metabolism as a single subject that encompasses the biosynthesis of all chemical structures. The lack of a theoretical basis for splitting metabolism into 'primary' and 'secondary' should be confronted, and a more robust evolutionary framework developed. The differences in various biosynthetic pathways do not arise because they follow different rules; rather the same rules apply to a different extent because they operate under different evolutionary constraints. These rules and constraints must be comprehended if a full understanding of metabolism is to be achieved and if attempts to control or change metabolism in organisms are to be successful.

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References

Boller T. 1995. Chemoreception of microbial signals by plant cells. *Annual Review of Plant Physiology and Plant Molecular Biology* **46,** 189–214.

Bu'Lock JD. 1973. Comparative and functional aspects of the isoprene pathway in fungi. *Pure and Applied Chemistry* **34,** 435–461.

Chapman DJ, Ragan MA. 1980. Evolution of biochemical pathways; evidence from comparative biochemistry. *Annual Review of Plant Physiology* **31,** 639–678.

Exton JH. 1994. Messenger molecules derived from membrane lipids. *Current Opinions in Cell Biology* **6,** 226–229.

Firn RD, Jones CG. 1996. An explanation of secondary product redundancy. In: Romeo, *et al*. *Recent advances in phytochemistry*. New York: Plenum Press, 295–312.

Firn RD, Jones CG. 2000. The evolution of secondary metabolism: a unifying model. *Molecular Microbiology* **37,** 989–994.

Hartmann T. 2007. From waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phyotchemistry* **68,** 2831–2846.

Hartmann T. 2008. The lost origin of chemical ecology in the late 19th century. *Proceedings of the National Academy of Sciences, USA* **105,** 4541–4546.

Hochachka PW, Somero GN. 1984. *Biochemical adaptation*. Princeton: Princeton University Press.

Horowitz NH. 1945. On the evolution of bichemical syntheses. *Proceedings of the National Academy of Sciences, USA* **31,** 153–157.

Jenks MA, Tuttle SD, Eigenbrode SD, Feldmann KA. 1995. Leaf epicuticular waxes of the *Eceriferum* mutants in *Arabidopsis*. *Plant Physiology* **108,** 369–377.

Jensen RA. 1976. Enzyme recruitment in evolution of new function. *Annual Review of Microbiology* **30,** 409–425.

Jones CG, Firn RD. 1991. On the evolution of secondary plant chemical diversity. *Philosophical Transactions of the Royal Society, Series B* **333,** 273–280.

Kaufman SA. 1993. *The origins of order: self organisation and selection in evolution*. New York: Oxford University Press.

Kössel A. 1891. *Archives of Analytical Physiology, Physiol Abteilung*, 181–186.

Mann J. 1987. *Secondary metabolism*. Oxford: Oxford University Press.

McConn M, Browse J. 1996. The critical requirement for linolenic acid is pollen development, not photosynthesis, in an *Arabidopsis* mutant. *The Plant Cell* **8,** 403–416.

Mothes K. 1960. Nebenwege des Stoffwechsels bei Pflanze, Tier und Mikrobe. *Mitteilungen Chemische Gesellschaft* **27,** 2–10.

Pogson B, McDonald KA, Truong M, Britton G, Della Penna D. 1996. Arabidopsis carotenoid mutants demonstrate that lutein is not essential for photosynthesis in higher plants. *The Plant Cell* **8,** 1627–1639.

Smith WL, Smith WL, Borgeat P, Fitzpatrick FA. 1991. The eicosanoids: cyclo-oxygenase, lipoxygenase, and epoxygenase pathways. In: Vane DE, Vane J, eds. *Biochemistry of lipids, lipoproteins and membranes*. New York: Elsevier, 297–325.

Vick BA, Zimmerman CD. 1984. Biosynthesis of jasmonic acid by several plant species. *Plant Physiology* **75,** 458–461.

Zeevaart JAD, Rock CD, Fantauzzo F, Heath TG, Gage DA. 1991. Metabolism of ABA and its physiological implications. In: Davies WD, Jones HG, eds. *Abscisic acid: physiology and biochemistry*. Oxford: Bios Scientific Publishers Ltd. 39–52.