The lost origin of chemical ecology in the late 19th century

Thomas Hartmann*

Institut für Pharmazeutische Biologie der Technischen Universität Braunschweig, Mendelssohnstrasse 1, D-38106 Braunschweig, Germany

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The origin of plant chemical ecology generally dates to the late 1950s, when evolutionary entomologists recognized the essential role of plant secondary metabolites in plant–insect interactions and suggested that plant chemical diversity evolved under the selection pressure of herbivory. However, similar ideas had already flourished for a short period during the second half of the 19th century but were largely forgotten by the turn of the century. This article presents the observations and studies of three protagonists of chemical ecology: Anton Kerner von Marilaun (1831–1898, Innsbruck, Austria, and Vienna, Austria), who mainly studied the impact of geological, climatic, and biotic factors on plant distribution and survival; Léo Errera (1858–1906, Brussels, Belgium), a plant physiologist who analyzed the localization of alkaloids in plant cells and tissues histochemically; and Ernst Stahl (1848–1919, Jena, Germany), likely the first experimental ecologist and who performed feeding studies with snails and slugs that demonstrated the essential role of secondary metabolites in plant protection against herbivores. All three, particularly Stahl, suggested that these ''chemical defensive means'' evolved in response to the relentless selection pressure of the heterotrophic community that surrounds plants. Although convincingly supported by observations and experiments, these ideas were forgotten until recently. Now, more than 100 years later, molecular analysis of the genes that control secondary metabolite production underscores just how correct Kerner von Marilaun, Errera, and, particularly, Stahl were in their view. Why their ideas were lost is likely a result of the adamant rejection of all things ''teleological'' by the physiologists who dominated biological research at the time.

herbivore | historical basis | plant protection | secondary metabolism

Chemical ecology refers to chemically mediated interactions between organisms and their biotic and abiotic environment. It covers a broad range of chemical interactions and signaling processes; major facets are (*i*) the chemical communication (chemical language, e.g., pheromones) of animals, particularly expressed in arthropods; (*ii*) the mutualistic interactions of organisms, e.g., plants and animals (pollination), plants and fungi (mycorrhiza), and plants and bacteria (symbiotic nitrogen fixation); (*iii*) the chemical defenses of organisms, e.g., plant defenses against herbivores and pathogens, animal defenses against predators and parasitoids, and microorganism defenses against food competitors; and (*iv*) protection against abiotic stress, e.g., plant defenses against damage by UV light, drought, or cold.

A major area of chemical ecology concerns the constant competition between the worlds of autotrophs and heterotrophs or simply plants and animals. During their evolution, plants have evolved sophisticated adaptations to cope with herbivores and pathogens while the latter developed similarly elaborated counter-adaptations to overcome plants' defenses. Plants produce a diverse array of metabolites that are not involved in primary metabolism. These secondary metabolites determine our sensory perception of unique characteristics of plants: We see the pretty colors and smell the fragrances of flowers and fruits, and we appreciate the distinctive tastes of spices, vegetables, and fruits. Moreover, all of the biological activities of plants that humans have used for medicinal reasons for hundreds of years can be attributed to secondary metabolites.

Entomologists in the middle of the 20th century were the first to rediscover the importance of secondary metabolites in plants' interactions with their environment. They emphasized the crucial role of secondary metabolites in host plant selection of herbivorous insects. In his classic paper, Gottfried Fraenkel (1) pointed out that secondary metabolites in plants function to repel or attract herbivorous insects. In the 1960s the newly reemerging field of chemical ecology began to prosper as the importance of plant secondary metabolites in the interactions of plants with their environment was grounded in measurement and observations (2). The field broadened to comprise all facets of chemically mediated organismic interactions; formal landmarks include the first book devoted to chemical ecology in 1970 edited by Sondheimer and Simeone (3), the publication of the first issue of the *Journal of Chemical Ecology* (www.chemecol.org/jce/jce.htm) in 1975 launched by Simeone and Silverstein, and the foundation of the International Society of Chemical Ecology (www.chemecol.org) shortly thereafter.

Fraenkel (1) mentioned Ernst Stahl, who in 1888 published comprehensive feeding experiments with herbivorous slugs and snails. Based on these studies, Stahl suggested that the various chemical protective means of plants were shaped and optimized under the selection pressure of the animal kingdom that surrounds the plants (4). Surprisingly, this experimentally well founded and convincing work was ignored for 70 years. Stahl is frequently quoted as an early pioneer of chemical ecology but rarely appraised in more detail (2, 5).

This article intends, first, to characterize the brief but flourishing period of early chemical ecology in the second half of the 19th century stimulated by Stahl and his contemporaries Anton Kerner and Léo Errera and, second, to address the question of what caused biologists for many decades to ignore and even reject these studies, despite their convincing ideas and results.

The Early Pioneers of Chemical Ecology: Characters, Facts, Ideas

In the second half of the 19th century, morphological and anatomical plant structures were almost exclusively interpreted in the context of the functional (physiological) needs of plants (6). Adaptations of morphological structures to environmental influences, for instance, the presence of thorns or spines to protect against browsing animals were either neglected or interpreted as a secondarily acquired advantage of an already existing structure. At that time, biologists began to go down one of two paths (7): "functional biologists" took an exclusively chemical approach to answer proximate questions about mechanisms, and "evolutionary biologists" asked historical questions about origins and the selective pressures

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*E-mail: t.hartmann@tu-bs.de.

that produced them. For a long time, there was little overlap and communication between these schools of biologists. Plant–animal interactions, which are characterized by physiological facts but based on evolutionary inferences, have a foot in both camps. The importance of the mechanical and chemical protective means plants take (*Schutzmittel der Pflanze*)[†] against animals and other environmental factors was recognized on and emphasized in several monographs and travelogues. Some of them are fascinating medleys of amazingly precise observations; others are examples of bizarre fancy, like Otto Kuntze's booklet (8). Among these naturalists the three above-mentioned pioneers stand out.

Anton Kerner von Marilaun. Anton Kerner von Marilaun was born in 1831 in Mautern (Lower Austria) and, early on, began exploring the flora of the Wachau. He studied medicine at the University of Vienna (1848–1854) but at the same time continued and intensified his botanical studies. At the age of 23 he became *Doctor Medicinae et Chirurgiae*. He practiced medicine for only 1 year and then realized his real desire, left medicine, and devoted himself to botany, accepting a position as a teacher in Ofen (Hungarian province). In 1860 he was appointed professor at the University of Innsbruck. The following 18 years in Innsbruck were the most productive in his life. During this time he declined offers from prestigious universities but did finally accept a position as Professor of Systematic Botany and Director of the Botanical Garden of the University Wien. In 1877, he was knighted and received the title *Ritter von Marilaun*. Marilaun was the name of his summer residence in Trins (Gschnitztal, Austria) where he built his own alpine research garden. Anton Kerner died in summer 1898 after a sudden stroke (for historical sources, see refs. 9 and 10).

Kerner started as a taxonomist. He explored the flora of almost all Austrian and Hungarian provinces and became the leading expert on Alpine flora (9). His intensive floristic studies in various distant geographic areas showed him the importance of geological, climatological, and historical factors on the appearance of plant species. These studies greatly influenced the direction of his research. He focused on the geographic distribution of plant species, classified vegetation units, and recognized correlations between plant distribution and climatic factors. These comprehensive studies are documented in one of Kerner's major monographs, *Das Pflanzenleben der Donauländer* (11). Kerner pioneered the emerging fields of phytogeography and plant sociology (9, 12). The variations among plant populations growing at different locations and under changing climatic conditions informed Kerner's evolutionary and ecological perspectives. In a 6-year, long-term experiment he cultivated >300 annual and perennial plant species in his four experimental gardens at different elevations and climatic conditions in Vienna [180 m above sea level (asl)], Innsbruck (569 m asl), Gschnitztal (1,215 m asl) and Blaser (2,195 m asl). After analyzing all morphological and phenological parameters, he reached this conclusion:

> Once seeds obtained from the alpine habitat were germinated in the Botanical Gardens of Vienna or Innsbruck, the developing plants immediately adopted the shape [Gestalt] and color corresponding to this habitat. Modification of shape and color caused by the changes of soil and climate are not retained by the offspring. The traits which represent these changes are not enduring.
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> Ref. 13, vol. II, p. 507 (my translation)

One of the first to clearly document environmental nonheritable changes in organisms, he also presented convincing arguments against Jean Baptist Lamarck's hypothesis of "the heritability of acquired characters," which was strongly supported at that time.

Kerner recognized not only that historical, climatic, and geological conditions greatly determine the distribution of plant species but also that biotic interactions have a crucial role. He performed elaborate studies involving insect-mediated pollination, seed dispersal, and plant protective means against herbivores and nectar robbers. Most of these ecological observations are documented in Kerner's famous textbook *Pflanzenleben* (13) [English edition (14)], which can be regarded as the first comprehensive survey of plant ecology.[‡] A monograph published in 1879 addresses the "protective means of flowers against unbidden guests" (16). A few selected examples should illustrate Kerner's contributions to the study of plant–insect interactions. Kerner emphasized the importance of mechanical defenses (thorns, spines, trichomes, etc.), chemically mediated defenses (alkaloids, essential oils, bitter compounds, saponines, coumarin, latex, etc.), and combinations of both (silicified or calcified cell walls and hairs, stinging hairs, glandular trichomes, etc.) for plant survival. He characterized the relationship between the animal and plant worlds not as warfare but as "armed freedom." Observing that certain animals feed only on specific plants—the larvae of the European peacock (*Inachis io*) on nettles (*Urtica dioica*), the larvae of the oleander hawk-moth on *Nerium oleander*, and the beetle *Haltica atropae* on leaves of the deadly nightshade (*Atropa belladonna*)—he realized that toxic plants may be poisonous for some animals but tolerated by others. Belladonna berries, for instance, are toxic for ruminant animals but harmless for many birds. He made thorough observations of various kinds of trichomes, glandular hairs, sticky girdles at stems (e.g., sticky catch-fly, *Viscaria vulgaris*), positioned strategically on plant organs (e.g., stems, pedicels, outer calyx) to prevent the visit of flower nectaries by "unbidden" small creeping insects that could disturb the efficient work of the real pollinators (16). With colorful descriptions he presented the amazingly diverse arsenal of sophisticated mechanical and chemical defenses of plants. Many of his examples have been forgotten and are no longer mentioned in current textbooks. According to Kerner's ideas, the evolution of plant adaptations to environmental selection pressure is based on spontaneous heritable variation and the subsequent selection of certain genotypes according to their competitive abilities:

> The so-called "adaptation" never is a direct one and never occurs as a result of a requirement. With other words: external conditions cannot provoke a heritable change of the gestalt, neither a beneficial nor an unfavorable, neither the formation of an element [Glied] nor its atrophy.
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> Ref. 16, p. 57 (my translation)

This opinion underscores how closely Kerner followed Charles Darwin's ideas (17). Both scientists had corresponded with each other and held each other in great esteem (10).

Léo Errera. Léo Errera was born in 1858 in Laeken near Brussels (Belgium). He studied systematic botany at the University of Brussels and received his diploma as *docteur en sciences naturelles* in 1879, after which he spent 3 years in Germany. In Strasbourg, he worked in the laboratories of the botanist Anton de Bary and the physiological chemist Felix Hoppe-Seyler. The latter encouraged his further biochemical projects. In Würzburg, Errera spent some time with Julius Sachs and studied plant physiology. In 1984, he returned to Brussels and founded the *Laboratoire d'Anatomie et de Physiologie Végétales*, which attained an international reputation under his direction. In 1905, just weeks after he had been elected president of the organizing committee of the next International

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[†] The term *Schutzmittel* includes all possible plant protective means, particularly mechanical defenses and protective compounds (*Schutzstoffe* or *Schutzexkrete*).

[‡] The term "ecology" (*Ökologie*) was introduced in 1866 by Ernst Haeckel (15), but it only slowly replaced the term "Biologie," which was used in the same sense in the 19th century.

Botanical Congress in Brussels in 1910, Errera died unexpectedly of a heart attack; he was only 48 years old (for historical sources, see refs. 18 and 19).

Errera was a versatile and amazingly productive scientist. His research included biological, systematic, physiological, chemical, and mathematical projects. He did pioneering work in two fields that are of particular interest to chemical ecologists: histochemistry and flower biology. Results in both fields stimulated Errera's interest in plant–animal interactions. Errera discovered the occurrence of glycogen in fungi and plants (amylopectin) by means of sophisticated histochemical methods. Later, he applied these techniques to the detection of alkaloids in plants and provided the first comprehensive picture of the tissue-specific distribution of this multifaceted class of secondary compounds in plants (20). The results are still relevant and impressively illustrate the validity and spatial resolution of his methods. Errera found that alkaloids are found (*i*) in cells, where they are separated from the cytoplasm and localized in the vacuole; (*ii*) in active tissues, i.e., close to meristems, in ovules, etc.; (*iii*) often in peripheral cell layers and trichomes of vegetative organs and fruits or seeds; (*iv*) around the vascular bundles in stems and roots; and (*v*) in the youngest cork cells and in laticifers when these are present. Based on these studies, he concluded (21)

> One gets the impression that in plants that produce relatively high amounts of alkaloids these emanate from a process targeting their formation.
>
> Ref. 21, p. 207 (my translation)

He emphasized the benefits for the producing organisms:

> Most alkaloid-containing plants are avoided by browsing animals. A few grams of alkaloids are equally efficient protective means as the most forceful thorns.
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> Ref. 21, p. 208 (my translation)

He also provided an evolutionary scenario for their origins:

> If alkaloids or analogously acting compounds are used as protective substances and natural selection comes into operation and gradually increases their production, this would entail the development of poppy, poison hemlock, deadly night shade and the whole array of poisonous plants and on the other hand also poisonous animals like toads, salamanders and snakes.
>
> Ref. 21, p. 209 (my translation)

Excited by studies on the cross-fertilization of flowers by insects and studies on plant structural adaptations to insect pollination (e.g., heterostyly in primroses), he published a paper on the effect of plant defenses against animals (22). With this paper, he hoped to motivate his botanist colleagues and amateur taxonomists not just to record plants in the field but to study their biology. The paper received much attention and initiated similar studies throughout Europe. Errera described field work carried out with his students, where he thoroughly documented the various ways plants can escape herbivory. A great number of plant species and animals (mostly mammals) were observed, and the various protective means were compiled. He summarized the possibilities plants have to protect themselves against animals in three categories:

A. General protection
1. Inaccessible habitats (in water, on rocks, along walls, etc.).
2. Inaccessible organs: crowns of high trees, rhizomes, bulbs, tubers, subterranean fruits, hidden entrances to nectaries.
3. Impenetrable hedges or thickets formed by social plants.
4. "Vassal plants" that are under the protection of certain animals (ant plants, mite plants) or protected by other plants (epiphytes, hedge plants, etc.).
5. Mimicry in the plant kingdom.

B. Anatomical (mechanical) protective means
6. Lignification, bark, cork, etc.
7. Organs that are tough, leathery, acute, sharp, calcified, silicified, spiny or sticky.
8. Thorns, spines, and stinging hairs.

C. Chemical protective means
9. Acids, tannins, etc.
10. Essential oils, camphor, etc.
11. Bitter-tasting fruits.
12. Glucosides.
13. Alkaloids.

Errera stressed that plant chemicals are as important as mechanical defenses. Displaying great foresight, he encouraged not only botanists to observe plant protection but also zoologists to study the counteradaptations (*les contre-adaptations*) of herbivores and did so at the time as the president of the Belgium Entomological Society encouraged his members to study insect–plant interactions. Errera expressed his hopes for the outcome off such studies as follows:

> We will clarify the details of the everlasting battle between herbivores and plants and understand the different stratagems—if it is allowed to call it this way—which are adopted for attack respectively defense by the hereditary enemies (ennemis héréditaires).
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> Ref. 22, p. 95 (my translation)

This may well be the first mention of an arms race between plants and herbivores.

Ernst Stahl. Ernst Stahl was born in 1848 in Schiltingheim (Alsace). He studied biology at the universities of Strasbourg and Halle, returning with Anton de Bary from Halle to Strasbourg, where he finished his doctoral thesis on the development and anatomy of lenticels and received his doctoral degree (PhD) in 1873. He continued his work in Strasbourg and began to study the development of lichens. He also spent some time in Julius Sachs' laboratory in Würzburg, where he accomplished his *Habilitation* (1877), after which he continued his research on phototaxis, chloroplast movement, and the physiology of high light and shade leaves for 3 years. In 1880, he was appointed professor in Strasbourg but 1 year later accepted a professorship at the University of Jena as Eduard Strasburger's successor. In Jena, Ernst Stahl found his scientific and social home; he stayed there for 38 years till his death in 1919.

Stahl's scientific activities in Jena included important physiological and ecological studies, such as excitability and chemotaxis of Myxomycetes, light-mediated processes in plants and light effects on leaf anatomy, the role of mycorrhiza, leaf movements, and ecological aspects of transpiration and assimilation. Detailed and informative appraisals of Stahl's personality and scientific work are found in a festschrift on the occasion of his 70th birthday (23). Two obituaries were written by his friends and colleagues Goebel (24) and Kniep (25).

Stahl was among the first scientists who performed experiments in ecology which is best illustrated by his studies on the defenses of plants against herbivores. Three publications address this topic: (*i*) the already mentioned comprehensive study, "Plants and Snails, A biological‡ study on the defensive means of plants against feeding damage by snails," published in 1888 (4); (*ii*) a shorter paper published 16 years later as a festschrift in honor of Ernst Haeckel's 70th birthday, titled "The defensive means of lichens against animal feeding damage" (26), in which Stahl expanded and completed his ideas; and (*iii*) Stahl's last publication, a comprehensive study of "The physiology and biology‡ of excretions" (27) in which, in addition to their physiological aspects, the ecological role of crystals and calcified tissues in plant defense is discussed.

Stahl's motto was "mein Laboratorium ist die Natur" ("my laboratory is nature"), which clearly finds expression in his working concept: "Feeding experiments in the laboratory stimulated by field observations tell us whether or not a plant is protected against a given animal species." Although he performed some studies with insects (e.g., grasshoppers and caterpillars) he used mostly slugs and snails in his experiments because they represent a class of formidable herbivores that are always abundant. The feeding studies included the following species of slugs, *Arion empiricorum*, *Arion hortensis*, *Arion subfuscus*, *Limax agrestis*, *Limax cereus*, and *Limax maximus*; and snails, *Helix pomatia*, *Helix hortensis*, *Helix nemoralis*, *Helix arbustorum*, and *Helix fruticum*. Stahl studied the behavior of these species in thorough field observations and compiled the feeding preferences of the individual species. He demonstrated, for instance, that many *Helix* species rarely fed on living plants but preferred dead or decaying plant matter, whereas *H. pomatia* and particularly the slugs *L. agrestis* and *A. empiricorum* fed on almost all living plants they could find. Stahl discovered that each species has its distinctive feeding preference, and this often changes during the season depending on the plants available. Stahl recognized the difference between generalist herbivores—he named them "omnivores"—and specialist herbivores. He confirmed in feeding experiments that omnivores refuse to feed on certain fresh plants but consume them voraciously after they are extracted with suitable solvents, for instance ethanol. On the other hand, he found that a specialist generally preferred its food plant in the native state and rejected or hesitantly fed on the respective extracted plant materials. Comprehensive observations and experiments with generalist and specialist herbivores led him to draw the following conclusions:

> On the same plant species omnivores and specialists behave diametrically differently.
> A "reciprocal adaptation" [a term he adopted from Otto Kuntze (8)] exists between a specialist herbivore and its host plant. Apparently both must coexist in a balanced equilibrium since the loss of its food plant would cause the extinction of the food specialist.
> A plant metabolite that acts as a deterrent for omnivores may be a feeding stimulant for a specialist herbivore.
> Only generalist herbivores are appropriate for use in feeding studies that ask whether or not a plant is chemically protected.

A typical feeding experiment was performed as follows: The plant was offered to a hungry herbivore. If the plant was not eaten, it was extracted with a solvent, for instance, hot or cold water or ethanol, dried, soaked in water, and offered again to the herbivore. If the material was eaten, two kinds of control experiment were performed: (*i*) The plant extract was re-added to the extracted plant material; (*ii*) oven-dried carrot slices were soaked in the plant extract and, in choice experiments, were offered together with water-soaked reference slices. In both control experiments, refusal of the herbivore to feed on the treated samples was considered as proof that the plant is chemically protected, at least against that herbivore. Using this strategy, Stahl performed many experiments with different generalist herbivores and a great number of potential food plants. He established that the following classes of secondary compounds were generally strong feeding deterrents: tannins, acidic cell saps (e.g., potassium bioxalate), essential oils, bitter-tasting compounds, oil bodies of liverworts, and various lichen acids and toxins.

In his later studies (26) Stahl demonstrated the protective role of lichen compounds against microbial attack in addition to plant-herbivore interactions. He described how lichen acids prevent lichen thalli from molding over weeks. He was probably one of the first scientists to demonstrate the antimicrobial effects of secondary metabolites under natural conditions.

As mentioned above, Stahl performed feeding experiments in the context of thorough field observations. His work in the field helped him recognize the great importance of mechanical means for plant protection in addition to chemical protection. He emphasized that chemical protection is often facilitated by mechanical structures, for instance, the stinging hairs of nettles or various stalked glandular trichomes. Purely mechanical protective means, such as trichomes and tough surfaces, may affect slugs and snails in different ways. They may prevent the herbivore from approaching the plant and starting to feed; or, if herbivores succeed in feeding, the intake of plant tissues may mechanically affect the soft tissue of their mouth parts. In experiments with prickly-haired plants (e.g., Boraginaceae), calcified trichomes (e.g., the file hairs of many Brassicaceae, which contain calcified bumps), and plants with silicified epidermal cells (*Equisetum* species, Cyperaceae, and certain grasses), Stahl catalogued these effects with the thoroughness with which he studied chemical protection. He confirmed that mechanically protected plant tissues are easily consumed after the mechanical defenses had been removed.

Two remarkable examples of almost-forgotten phenomena combining chemically and mechanically mediated protection should complete this short survey of Stahl's multifarious studies. Stahl discovered that some species of the Onagraceae (*Oenothera* sp., *Epilobium hirsutum*, and *Circaea lutetiana*) have their stems covered with what he called "acid hairs," which at their apex exude droplets of acidic fluid. This acidity can easily be tasted with the tongue or demonstrated by touching the tissue surface with pH paper. If the acidic excretion is mechanically removed or washed off by rain, it is regenerated within a few hours. He observed that snails and slugs never attacked plants protected by acid hairs. To my knowledge, this simple and impressive phenomenon is not mentioned in any existing botanical text books or monographs on plant trichomes (28, 29). The second example concerns the role of raphides, which are bundles of numerous calcium oxalate needles. Raphides occur in phylogentically distant plant taxa, such as Rubiaceae, Vitaceae, and Onagraceae as well as many monocot families. Raphids, located in single longish cells, are always associated with mucilage. If a raphid-containing cell is damaged, for instance, by herbivore attack, raphide needles, facilitated by mucilage, are squeezed out through the top ends of the cells, the walls of which are often thin. The needles easily penetrate the soft skin of an herbivore's mouthparts. The acrid and irritating taste of leaves of *Arum maculatum* or other raphide-containing plants is not caused chemically, as often stated, but mechanically, a result of injury by raphide needles. (Stahl confirmed this in a self-experiment with isolated pure raphide needles.) All plant materials containing raphides remain untouched by slugs and snails. Stahl detected the protective function of the raphides accidentally in the course of feeding studies. Snails rejected raphide-containing leaves extracted with various solvents to test the possibility that a chemical defense was involved. When, however, the calcium oxalate needles were dissolved by treating the leaves with hydrochloric acid, the treated leaves were readily eaten. Again, this exciting plant defense strategy, although briefly mentioned in older textbooks (6), is rarely mentioned today.

Stahl also studied the tissue distribution and appearance of protective chemicals during plant development. In accordance with Errera's histochemical alkaloid studies, he found that protective compounds are preferentially concentrated in peripheral tissues and often found in tissues close to meristems. He found that inflorescences are often better protected than vegetative tissues and young leaves better than old leaves. He noticed that tannin cells, oil cells, glandular trichomes, and raphides appear early during tissue development and are already present in their final number and concentration in very young leaves and that these defenses move apart or are diluted during leaf elongation.

Based on his comprehensive field observations and laboratory experiments, Stahl concluded that the various plant defenses provide relative but never absolute protection. He observed great variation in the efficiency of the various defenses against the different herbivores he studied, and he marveled at the rich diversity of mechanically and chemically mediated defenses in plants. Plant tissues that are easily amenable to slugs and snails are usually chemically well protected, whereas tissues that hamper herbivores mechanically are chemically less actively protected.

Stahl recognized that many morphological structures of vegetative and reproductive plant organs are understandable only from the point of view of plant–animal interactions, for instance, the relationship of flower shape to pollinators. He suggested the existence of analogous relations between the diversity of plant protective means and herbivores. He described the evolutionary scenario as follows:

> Here the objection has to be countered that substances like tannins, bitter compounds, essential oils, alkaloids, etc.—whose role in plant chemistry is almost completely obscure—would exist as essential components of metabolism in a complete absence of animals. That these compounds are essential components of metabolism should not be denied as well as their presence in plants before they were subjected to natural selection by herbivores. However, their current quantitative design, their distribution within plant organs, their often preferred peripheral accumulation, and, particularly, their early appearance can exclusively be understood by the impact of the animal kingdom surrounding plants. Moreover, even the idea should not be denied that the quality of all these compounds in respect to smell, taste, toxicity and thus chemical composition must be affected by the selection pressure of the animal kingdom. The variability of plants not only concerns morphology but also metabolic processes. Humans obtained by breeding of inconspicuous, tasteless wild fruit species—I just recall the pears—a rich variety of differently smelling fruits which at least in respect to their aroma have different chemical composition. It can be assumed that in the same way under the selection pressure of herbivores, plant constituents with improved deterrent or detrimental properties against herbivores are created.
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> Ref. 4, p. 566 (my translation)

It is remarkable that Errera and Stahl, and basically Kerner, have had the same evolutionary visions: By-products, such as alkaloids, essential oils, and tannins, that are "metabolically useless" (Errera) or of "unknown functions" (Stahl) provide the raw materials that had been qualitatively and quantitatively optimized as plant chemical protective means under the selection pressure of herbivores. Inspired by Charles Darwin, they adopted his theory of natural selection (17). Today it appears incomprehensible that the convincing view of these great scientists had been almost completely neglected for 70–100 years.

Early Chemical Ecology: Why Neglected and Forgotten for Decades?

The ideas about the protection of plants against herbivores raised by Kerner, Errera, and Stahl caused almost unanimous skepticism among their contemporaries. As is often the case for scientific controversies, the facts were not disputed, but their interpretation was controversial. The idea that plant defenses have been shaped by the selection pressure of the animal kingdom was rejected as being teleological. Functional biology (physiology), which dominated the research agenda of biology till modern times, was critical of the teleological approach. Each process suspected of being "goal-directed" (e.g., plants protect themselves by producing chemical defense) was rejected. At that time, the existence of complex genetic programs mediating teleonomic (i.e., program directed) traits was unknown: "A teleonomic process or behavior is one which owes its goal-directedness to the operation of a program" (E. Mayr) (7). A process like natural selection operates on a strictly *a posteriori* basis, unlike a teleological process, which sees plan and design in nature. A few examples illustrate the stigma attached to teleological interpretations in the beginning 20th century. The third edition of Kerner's *Pflanzenleben* edited by Adolf Hansen (30) appeared 23 years after Kerner's death. Hansen changed as little as possible to retain Kerner's unique diction but felt obliged to insert the following comments in the chapter dealing with plant chemical defenses:

> If now many plants are protected against herbivory by self synthesized compounds it may be questionable to call these protective compounds with the implication that plants generate these compounds to protect themselves.
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> Ref. 30, vol. 1, p. 116 (my translation)

In his well known textbook *Physiological Plant Anatomy* (6), five editions of which appeared between 1884 and 1918, Gottlieb Haberlandt included results from some of Stahl's experiments, but fewer and fewer from edition to edition. In the last edition, he introduced Stahl's raphide story with the qualifier: "Even if Stahl may have overestimated the protective role of raphides. . . ." Haberlandt distinguished between physiological adaptations, which result from appropriate morphological structures and reflect the economy of plant function, and biological (ecological)[‡] adaptations. The latter are always of secondary importance. In the case of the raphides and chemical defenses, Haberlandt wrote:

> Oxalic acid is a common by- and end-product of metabolic processes in plants. It is toxic as free acid but nontoxic as insoluble calcium oxalate crystals. These crystals often have the shape of spears or needles and thus are suited to function as mechanical protection means against herbivorous insects and snails. In the same way other metabolic end-products may have secondarily attained ecological importance.
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> Ref. 6, pp. 6–7 (my translation)

This typical explanation of the ecological functions of plant metabolites could have been found in almost any textbook of plant physiology until the 1980s. Even Errera apparently changed his mind and, 17 years after publication of his classic article (20), adopted the common opinion and avoided any evolutionary interpretation of ecological functions of alkaloids:

> Granting that the physiology of alkaloids is far from settled, I think a critical study of their topography as well as their behavior in germination, growth, etiolation, maturation of seeds, etc., supports the view that they are waste-products, resulting from the catabolism of cytoplasm, and secondarily used for defense against animals. A few grams of an alkaloid constitute a protection not less efficient than the strongest spines.
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> Ref. 31, p. 187

The characterization of plant chemicals as metabolic waste-products that in some cases may have secondarily acquired ecological functions survived in plant biology well into the second half of the 20th century. The idea that plant secondary metabolites were waste-products was supported by plant physiologists and phytochemists. A few historical landmarks illustrate this view. Interestingly, Julius Sachs and Wilhelm Pfeffer, the founders of modern plant physiology, defined what later was referred to as "plant secondary metabolism"[§] without using the term "waste-products."

Sachs wrote

> We can designate as by-products of metabolism such compounds which are formed metabolically but which

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[§] The term "secondary metabolites" was coined by the biochemist Albrecht Kossel (32) in 1891 to characterize cell components that contrast with "primary metabolites" and are not found in any developing cell. The term was adopted by Friedrich Czapek in his *Biochemistry of Plants* (33) and has been used ever since.

> are no longer used in the formation of new cells. . . . Any importance of these compounds for the inner economy of the plant is so far unknown.
>
> Ref. 34, p. 641 (my translation)

Pfeffer stated

> In contrast, many other compounds such as alkaloids, glycosides, etc. are obviously aplastic constituents which are largely created for ecological purposes.¶
>
> Ref. 35, vol. 1, p. 454 (my translation)

For Sachs's clear definition to still be valid, we only have to substitute "secondary products" for "by-products" and "primary metabolism" for "inner economy." Sachs did not address functional aspects. Remarkably, 24 years later, Pfeffer adopted Stahl's view. However, his textbook was probably the only one published approximately over the next 80 years that emphasized the ecological function of plant secondary metabolites. Until the middle of the 20th century, research on plant secondary metabolism dealt almost exclusively with the flourishing field of natural product chemistry. Because of work in this field, we have an enormous array of diverse chemical structures (some 200,000 are known so far), many of them with interesting biological and pharmacological activities and great economic importance. Physiological studies on plant secondary metabolism began in the early 1950s with tracer feeding experiments; these were followed by the enzymatic characterization of biosynthetic pathways. In the mid-1980s it became clear that secondary metabolites are *de novo* synthesized from simple precursors of primary metabolism through elaborate sequences of reactions catalyzed by specific enzymes (for review, see ref. 36 and references therein). This knowledge relieves secondary metabolites of their image as waste-products. Among plant physiologists, secondary metabolism is now regarded as an essential part of metabolism, even without indicating any particular function. As already pointed out, the ecological role of plant secondary metabolism in Stahl's evolutionary context was given new life by evolutionary entomologists in the 1960s. These ideas were, however, accepted only slowly by plant biologists (37, 38). Among the pioneers of the so-called new chemical ecology within plant science were Tony Swain, who in the 1970s fought against the waste-products lobby (39), and Jeffrey Harborne, author of a well known textbook (40). However, the most progress in the rapidly developing field was provided by entomologists, who emphasized the essential role of plant secondary metabolites in plant–insect interactions in an evolutionary context (for review, see ref. 2 and references therein).

The rapid progress in molecular biology during the past few decades has enforced the unification of the ideas of functional and evolutionary biology in plants. In fact, plant biologists now broadly accept the essential ecological role of secondary metabolism. During the last 10 years, scientists have had fascinating insights into the evolutionary creation of genetic diversity of secondary metabolism. The evolution of new genes by duplicating the genes of primary metabolism, followed by the functional diversification or new functionalization of the duplicates under the selection pressure of the environment (41, 42), has been demonstrated for key biosynthetic enzymes of a number of classes of secondary metabolites, such as terpenoids (43), polyketides (44), phenolic esters (45), benzoxazinone alkaloids (46), and pyrrolizidine alkaloids (47). In conclusion, we see that the evolutionary ideas of Stahl and his contemporaries are confirmed by recent concepts of molecular evolution of plant secondary metabolism. After a long period of dormancy, the ecological role of plant secondary metabolism in interactions between plants and a mostly hostile environment that was first suggested by a few pioneers in the late 19th century finally seems to be broadly accepted.

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¶Pfeffer distinguishes between "plastic" and "aplastic" metabolites, which correspond to "primary" and "secondary" metabolites, respectively.

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