Contemporary and future studies in plant speciation, morphological/floral evolution and polyploidy: honouring the scientific contributions of Leslie D. Gottlieb to plant evolutionary biology

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Introduction

Contemporary and future studies in plant speciation, morphological/floral evolution and polyploidy: honouring the scientific contributions of Leslie D. Gottlieb to plant evolutionary biology

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1. Introduction

This special commemorative issue is dedicated to the life and scientific contributions of Leslie D. Gottlieb. Dr Gottlieb's contributions to our understanding of plant evolution were many and varied, from which we selected some of the most influential to be included in this special issue honouring his career. Gottlieb's studies were largely hypothesis driven, and he invariably spelled out the rationale for the design of his research and how the results could be used to test his hypotheses. His research was of general interest and attracted widespread attention because it focused on central issues in plant evolution. Though Gottlieb used applicable state-of-the-art methods for that time period, the tremendous methodological and technical advances since his work, especially in the areas of molecular biology, genetics and now genomics, make it possible to examine in greater depth the questions that most intrigued him. The papers in this special issue, contributed by leading workers using largely cutting-edge methods, document that Leslie Gottlieb's work was indeed visionary and that it remains as relevant today as it was when published (figure 1).

It could be argued that the process of speciation is central to any discussion of plant evolution because the tremendous diversity seen in the green plant clade is the result of the generation of independently evolving lineages. Interest in and research on speciation has been centred on primary divergence and the processes that drive the divergence [1–7]. However, it is now increasingly recognized that hybridization between divergent taxa at the same ploidal level can generate novel, independent lineages, a process termed homoploid hybrid speciation [8,9]. Similarly, recent analyses have convincingly demonstrated that *all* land plants are 'palaeopolyploid' and that all angiosperm lineages contain multiple rounds of past genome doubling [10], and it has long been recognized that polyploidy is an important mechanism for hybrid speciation within contemporary plant lineages [11]. As a result of its pervasiveness in plant evolution, polyploidy receives considerable attention in this issue, just as it did in Leslie Gottlieb's research.

The evolutionary shift from outcrossing to selfing is generally recognized as one of the most frequent transitions in flowering plants [12,13]. Among many other things, it may serve as an isolating mechanism [14–16], and indeed in many plant lineages the origin of self-compatibility with the loss of function of the self-incompatibility locus is associated with speciation [16–19]. In fact, Leslie Gottlieb provided an elegant example of the association of speciation and the transition to selfing in his classic studies in the genus *Stephanomeria* [20,21]. It had long been recognized that selfing species are less variable genetically at
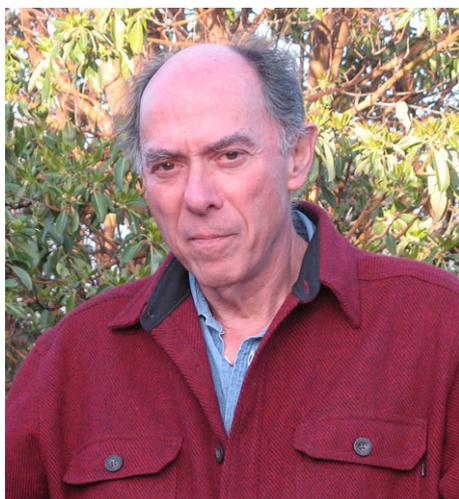
Figure 1. Leslie D. Gottlieb (1936–2012). Photo taken in Ashland, Oregon on 25 May 2009, one day before his 73rd birthday. Photo courtesy of Vera Gottlieb. (Online version in colour.)

the population level [22], and as discussed by Barrett *et al*. [23] in this issue, Gottlieb was one of the first to use enzyme electrophoresis to document lower genetic diversity in outcrossing compared with selfing plants [20,24–26].

High-throughput DNA sequencing now makes it possible to examine a number of long-standing questions about the factors that drive the transition to selfing and the consequences of its evolution. In this issue, Barrett *et al*. [23] examine the efficacy of genomic data to test the two most frequently cited hypotheses for the evolution of selfing. The first is the so-called automatic selection hypothesis in which a selfer can contribute outcross pollen as well as fertilize its own ovules (3 : 2 advantage) [27,28]. The second major hypothesis for the evolution of selfing is reproductive assurance, which posits that selfing will be favoured when pollen vectors and/or compatible mates are rare and limit seed production [29,30]. Inbreeding depression is recognized as the most important factor opposing the evolution of selfing.

Barrett *et al*. [23] provide a test, first advanced by Schoen *et al*. [31], for inferring the importance of the two hypotheses for the origin of selfing. The rationale of Schoen *et al*. [31] for using molecular data to test the two hypotheses is that reproductive assurance would involve population bottlenecks, and possibly founder events, both resulting in reduced genetic diversity throughout the genome. In contrast to reproductive assurance, with automatic selection, selfing mutants have a 3 : 2 advantage and could spread through a population in the absence of population bottlenecks. Under this scenario, there would not be reduced diversity at unlinked neutral loci. High-throughput sequencing technologies now allow assessment of genome-wide diversity in a comprehensive manner not even dreamed of when Leslie Gottlieb was employing enzyme electrophoresis for measuring diversity at relatively few loci. Using comparative empirical data and simulations, Barrett *et al*. [23] suggest that available molecular data are not able to test hypotheses for the selective forces or mechanisms that drive the transition to selfing. While they find little evidence of bottlenecks associated with the transition to selfing, they emphasize that this does not rule out the role of reproductive assurance as a driver of the evolution of selfing. Also, it is possible that genetic bottlenecks could occur subsequent to the origin of selfing. A critical factor in using molecular or other

data for elucidating factors promoting selfing is studying plants that are in the process of the transition or where a transition from outcrossing to selfing has been very recent. Indeed, Gottlieb [20] emphasized that studying recent divergence is critical for understanding the forces and mechanisms in plant evolution. Barrett *et al*. [23] highlight the importance of future studies integrating data from demography, genomics and experimental populations for identifying the drivers of mating system shifts, including the role of these shifts in facilitating divergence and speciation.

Speciation has traditionally been viewed within a geographical context and the biogeographic/spatial scales on which it may occur continue to be a topic of discussion and debate. As a result, the geographical context of speciation is a component of several papers in this issue, just as it was a topic that permeated many of Leslie Gottlieb's scientific papers. As stated by Coyne and Orr [32, p. 4] 'Among all the scientifically tractable questions about speciation, the most hotly contested concerns its biogeography'. Allopatric speciation has been historically the most widely accepted geographical mode of speciation because divergence between geographically separated populations may occur through time without being retarded or prevented by gene flow and recombination. By contrast, sympatric homoploid speciation has been the most contentious mode of speciation, with arguments against its occurrence centring on several issues. One problem is the challenge of documenting whether divergence occurred in sympatry, or divergence was in fact allopatric with subsequent secondary contact [33–35]. While it is difficult to rigorously falsify the allopatric–secondary contact hypothesis, evidence such as sister group relationship and high genetic similarity between the species in question [20,36], especially when the species occur together at one locality or in a restricted geographical area, combine to argue strongly for *in situ* divergence [20,33,35]. Leslie Gottlieb was very interested in sympatric speciation. One of his earliest and most highly cited papers [20] contained 'sympatric speciation' in the title and focused on divergence within a local population.

As indicated above, another major question regarding sympatric divergence is how it could occur in the face of gene flow from the parental species. Data from molecular markers and ecological studies show the potential for selection to drive divergence in the face of gene flow and recombination [37–41]. In his studies of *Stephanomeria*, Gottlieb was concerned with how initial divergence could have occurred and how the rarer derivative species could coexist with its progenitor at the same locality. While he identified several factors reducing interspecific gene flow and thus presumably explaining the rarity of natural interspecific hybrids in *Stephanomeria*, he never succeeded in elucidating the factors (indeed, if they existed) contributing to ecological/habitat divergence between the two species growing intermixed at a single locality [20,21,42,43]. The same issues that intrigued Gottlieb decades ago remain of interest and the focus of contemporary studies.

In this issue, Papadopulos *et al*. [44] continue a series of studies [33,35,39,40] to test the hypothesis of sympatric speciation using plants of Lord Howe Island as a model system. They employ molecular markers to determine genetic structure (and thus infer gene flow) associated with several ecological factors (both individually and in combination), community composition and spatial scale to infer which of these variables may be important in divergence. Papadopulos *et al*. [44], while providing the appropriate caveats for the approaches they

employed, present data supporting ecological divergence as a common factor associated with genetic divergence within species on Lord Howe Island.

Future studies of sympatric speciation will focus on fine-scale genetic analyses of the traits, e.g. adaptation to edaphic types, flowering time, selfing, etc. that lead to divergence, even in the face of gene flow [45,46]. These genetic/genomic investigations will be most powerful when combined with field studies such as transplant experiments [47–49]. The ultimate goals of such studies would be to demonstrate that plants with certain phenotypic traits are more fit in a given environment than those with alternative traits, and then eventually to elucidate the genetic basis of the contrasting traits. Species on Lord Howe Island appear to be excellent candidates for these types of intensive studies.

Leslie Gottlieb's interest in studies of speciation at the local level via the divergence of populations within the ranges or on the periphery of geographically widespread species extended beyond his classic investigations of *Stephanomeria malheurhensis* [20,21], and included members of the genera *Layia* [50] and *Clarkia* [51]. Those investigations consisted of morphological, biosystematic and electrophoretic approaches. In the case of *Layia*, the localized species is an edaphic endemic, whereas in *Clarkia* habitat differences between the two species were not apparent and, as noted earlier for *Stephanomeria*, were never well characterized.

The contribution by Ferris *et al.* [52] in this issue has a strong geographical component and focuses on population divergence within the range of one species to give rise to a new species. This geographical scale of speciation is receiving considerable attention with the growing appreciation that it may be more prevalent than previously recognized [53,54]. The genus *Mimulus* (monkeyflowers) has attracted the attention of many researchers and has attained 'model' status for plant evolutionary studies because of its array of ecological, phenotypic and genomic diversity [55]. Within *Mimulus*, the geographically widespread, morphologically variable, outcrossing and ecologically diverse *Mimulus guttatus* complex has proved rewarding for studies of local speciation. Within the geographical distribution of the complex, there are localized (commonly on specialized substrates), often selfing, populations that can be delimited morphologically, albeit with some effort in certain cases, and have been recognized as species in many instances [54,55]. These localized species have diverged independently from within the *M. guttatus* complex and represent multiple progenitor/derivative species pairs [56].

Ferris *et al.* [52] studied the recently described *Mimulus filicifolius*, a species in the *M. guttatus* complex. They employed several molecular markers to demonstrate that the species is genetically distinct, and thus presumably not just a minor morphological variant of its closest relative, another localized derivative species in the *M. guttatus* complex. Ferris *et al.* [52] then studied components of reproductive isolation. They found edaphic differences between *M. filicifolius* and its presumed closest relatives. These differences include soil temperature, per cent moisture and saturation point. Molecular markers showed that *M. filicifolius*, like some other local *Mimulus* species, is highly self-fertilizing [54]. As discussed earlier, selfing can serve as a barrier to gene flow among populations and facilitate divergence and speciation. Lastly, Ferris *et al.* [52] demonstrated strong post-zygotic isolation between *M. filicifolius* and *Mimulus laciniatus*, another local species and presumably the closest relative of *M. filicifolius*.

The genomic resources available for *Mimulus* (PHYTOZOME v. 8.0; www. phytozome.net) are providing finer insights into the genetic architecture of traits that drive divergence and speciation. Two recent investigations illustrate the value of genomic tools for examining local speciation, and point to additional approaches that could be applied, for example, to elucidate the genetic architecture of isolating barriers detected by Ferris *et al.* [52]. One of these studies [57], when taken within the context of prior investigations, provides an elegant example of how ecological and genomic studies may be complementary in examining early stages in local population divergence and incipient speciation. The species *Mimulus aurantiacus* has red- and yellow-flowered ecotypes, with the former almost exclusively hummingbird pollinated and the latter pollinated primarily by hawkmoths [58,59]. The isolation of the ecotypes is not complete, as hybrid zones occur where species overlap [60], and there are weak intrinsic barriers to gene flow between the two ecotypes [61]. These observations indicate that selection for different pollinators of the two floral colour morphs is an important factor in initial divergence. Therefore, elucidating the genetic architecture of flower colour would provide clues to the genetics of incipient speciation. Streisfeld *et al.* [57] demonstrated that flower colour differences between the two ecotypes of *M. aurantiacus* are based on a *cis*-regulatory mutation in a transcription factor that regulates enzymes in the anthocyanin biosynthetic pathway. The two ecotypes are fixed for alternative alleles specifying the floral colour morphs. Fixation of the alleles has occurred despite gene flow between the two ecotypes at neutral molecular markers, indicating that selection has driven the fixation of the alternative alleles [57].

Another recent study involves *M. guttatus* and one of its derivative species localized on high-copper soil from old mines in California [62]. Prior studies [63,64] suggested that copper tolerance and hybrid lethality are controlled by a single locus because crosses between copper-intolerant and copper-tolerant plants resulted in various levels of lethality in the F_1 hybrids. However, high-resolution genome mapping demonstrated copper tolerance and hybrid lethality are controlled by two tightly linked loci and that copper tolerance is not pleiotropic with hybrid inviability [62]. These two recent studies [57,62] demonstrate not only the utility of cutting-edge approaches for elucidating the genetic basis of characters, but, perhaps even more importantly, the enhanced value of the data when applied to interesting evolutionary situations/questions identified and posed by earlier workers using field observations and biosystematics approaches.

In contrast to the universal recognition of primary divergence in plant speciation, the importance of hybridization in adaptive evolution and in plant speciation at the same ploidal level has been more debateable [65–67]. With the advent of the Modern Synthesis, there was a general divide between those studying animals and plants, with the former viewing hybrids as maladaptive and hybridization as a final step in the development of reproductive barriers through the process of reinforcement [65]. On the other hand, prominent plant evolutionary biologists, with very few exceptions [66], tended to view hybridization as a creative process [67,68]. While the role of hybridization and the formation of maladaptive hybrids in plants as 'reinforcement' in speciation is of current interest [69], it is also now well accepted that hybridization functions in a creative way in plant evolution [9,11,70].

The contribution by Abbott & Brennan [71] focuses on hybrid zones along altitudinal gradients as model systems

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for studying the evolutionary role of hybridization. Assessing the impact of hybridization involves determining the fitness of hybrids relative to their parents, identifying the traits that affect fitness and elucidating the genetic basis of those traits [72–74]. Hybridization may create novelty, including new species [8,9,71,75,76], and also be a mechanism transferring adaptive traits between species [77,78], and in these two ways be important in plant evolution. Abbott & Brennan [71] emphasize the challenges of understanding the evolutionary impact of hybridization and multi-faceted studies, including field, experimental and genetic/genomic approaches, will be needed in the future to meet those challenges [46–48].

Homoploid hybrid speciation, the stabilization of hybrid recombinants (typically between two species) at the same ploidal level, has been the subject of renewed interest and activity over the past two decades, due largely to the work of Rieseberg and collaborators on the sunflower genus *Helianthus* [8,79–83]. Plant evolutionists have been interested in the possibility of homoploid hybrid speciation for decades, with models proposed [84,85], stabilized hybrids synthesized [86–88], and a handful of reports of naturally occurring hybrid species based on data from morphology and biosystematics [89]. Only about 20 cases of hybrid species in nature have been reported [11] but the number varies depending on what is considered sufficient evidence for documenting hybrid origin in any given case. Whether the difficulty of detection or true rarity are more significant causes of the paucity of well-documented examples of naturally occurring homoploid hybrid species remains an open question, and it could in fact be a combination of the two factors. This is not a trivial issue because rigorous documentation of hybrid origin is obviously the critical first step in any study of homoploid hybrid speciation. Leslie Gottlieb was the first to employ molecular markers to document the occurrence of a naturally occurring stabilized homoploid hybrid species [90]. With genomic resources providing unlimited molecular markers, it will become more straightforward to document hybridization [91].

Homoploid hybrid speciation is generally regarded as a form of sympatric speciation [92] initiated by gene exchange between two co-occurring species, and indeed this is likely the common condition. However, whether or not the initial hybridization occurred between sympatric congeners, or via some other mechanisms such as rare dispersal events, is not always easy to discern. Even if hybrids originate initially from crosses between sympatric parents, it does not mean that the stabilized hybrid derivatives would necessarily evolve in sympatry with the parental species [92]. Indeed, one of the classic examples of a homoploid hybrid species, *Senecio squalidus* [93,94], evolved and stabilized in England following the introduction of material from a hybrid zone in Italy. This is an example of a sympatric origin of a homoploid hybrid species, but with stabilization of the species in allopatry with its parents.

Studies of homoploid hybrid speciation will become increasingly multi-faceted. In addition to employing large suites of molecular markers or tools such as genomic resequencing to document hybridization, field and experimental studies of the fitness of parents and hybrids under different habitats and experimental manipulations [95,96] will be expanded. The real frontier will be in using genomic tools for achieving refined insights into the processes facilitating the isolation and stabilization of the hybrid derivatives. One focus will be on intrinsic isolation of parents from hybrids by selection for fertile genetic and chromosomal recombinants of parental karyotypes in the hybrids [81–83]. A second major focus will be elucidating the genetic/genomic basis of adaptive ecological/phenotypic traits in the hybrid species [97]. Resequencing data promise insights into these questions, as well as many others, such as the genomic distributions of parental contributions and the genetic architecture of adaptive traits.

Transposable elements (TEs) constitute the predominant portion of most plant genomes [98]. Given their ubiquity, comparative studies of TE activity and proliferation in hybrid species and their progenitors are potentially relevant to understanding the speciation process. Determining their role in the stabilization of hybrid genomes and the divergence of parental and hybrid genomes are areas of interest. The same studies could also reciprocally illuminate the dynamics of TE activation and proliferation in plant genomes. Returning to hybrid sunflowers, an initial study [99] showed that the larger genomes of hybrid species compared with their diploid parental species was due primarily to TEs (more specifically, long terminal repeat retrotransposons). In this issue, Renaut *et al.* [100] continue studies [101,102] into the dynamics of TEs in two parental *Helianthus* species and their homoploid hybrid derivative species, and use high-throughput technology to assess transcriptional activity of TEs. The authors found higher mean values of aggregate TE expression phenotypes in the hybrid species compared with the parental species or F_1 hybrids between the parents. They detected variation within species in both the aggregate levels and the component elements making up the total levels. The reason or reasons for the variation remain obscure and the authors suggest several possibilities and future studies to address the questions.

The role of TEs in hybridization and hybrid speciation is an important question because, as emphasized by Renaut *et al.* [100], of the necessary balance between the possible negative impact of proliferation of TEs on fitness, on the one hand, and their role in the reorganization and stabilization of hybrid genomes and the origin of phenotypic novelty on the other hand. The fact that all of the hybrid sunflower species have elevated TE proliferation relative to their parents and synthetic F_1 hybrids suggests that past bursts of TE activity play some role(s) in the success of the stabilized hybrid derivatives. Identifying the mechanisms that regulate and repress the expansion of TEs in hybrids and the possible phenotypic effects of the resulting insertional mutagenesis will remain an active area of research.

Leslie Gottlieb, while disposed to using available molecular methods in his studies, always kept in mind that it is phenotypes and their underlying genetic architecture that are visible to natural selection. Thus, often in collaboration with Vera Ford Gottlieb, he studied the genetic/developmental aspects of both floral and vegetative morphology [103–108]. One example serves as an illustration of how their work stimulated further research. They were intrigued by the genetic basis and ecological/evolutionary significance of petal spot patterns in the flowers of a species of *Clarkia* [105], a genus they used as a model system for many and varied studies. Subsequent studies have examined the ecological significance [109,110] and genetics [111] of petal spot patterns.

The contribution to this issue by Olsen *et al.* [112] on cyanogenesis (the production of hydrogen cyanide in damaged plant tissue, which in turn functions to reduce the activity of herbivores) in the genus *Trifolium* (clovers) is illustrative

of the multi-faceted studies valued by Gottlieb for understanding the genetic basis of phenotypic traits and the ecological significance of the traits. Olsen et al. [112] delve into not only the genetic architecture of the trait of interest, but also place it in an evolutionary context. Two key questions are whether the same traits have evolved more than once within a clade, and if so, whether the same mechanism was recruited to achieve parallel evolution of the trait. Olsen et al. [112] demonstrate how molecular data can be employed both for dissecting a trait and for tracing the evolutionary history of the trait. They show the power of genetic/genomic data for elucidating the mechanisms for cyanogenesis, but also emphasize that identifying the ecological factors shaping the patterns of occurrence seen in nature is a daunting task. This point is especially well illustrated in the cyanogenic response, which comprises two distinct components, synthesis of cyanogenic glucosides and the subsequent production of hydrogen cyanide. In some Trifolium species, cyanogenic glucosides are produced, but hydrogen cyanide is not. This situation requires the formulation of more complex adaptive hypotheses than the more straightforward situation where the herbivore-deterring hydrogen cyanide is produced. The authors consider several scenarios that have been advanced under which cyanogenic glucosides in the absence of conversion to hydrogen cyanide could be adaptive. The results of Olsen et al. [112], along with the papers they cite, provide an excellent example of the complexity of the ecological component of ecogenomic [113] studies, something that has been emphasized by others [46–48].

The diversity of flower structure and colour have long-fascinated biologists, with a large component of the interest centred on the ecological roles of floral attributes in pollination and other aspects of reproductive biology [114]. The papers by Hileman [115] and Wessinger et al. [116] consider floral evolution at different taxonomic scales, the former taking a broad perspective of floral symmetry in angiosperms, and the latter focusing on two species in the genus Penstemon. Gottlieb's interests and published work have significant elements in common with the topics of both papers. As pointed out by Hileman [115], Leslie Gottlieb was interested in floral development before the age of genomics and the evolution of development (evo-devo), and this interest is documented in several of his publications [104–108]. Also, he strived to place floral transitions in an evolutionary context [106,107], although some of his early work predated the routine use of DNA sequences for constructing phylogenetic hypotheses. The current availability of genetic/genomic tools now makes it possible to study the developmental genetic basis of floral diversity, and the construction of phylogenetic hypotheses from genetic/genomic data makes it possible to view the origin of diversity and the relative timing of transitions in floral traits [115,117,118].

Floral symmetry has long received attention from taxonomists, phylogeneticists and ecologists because of its utility as a character in systematics and phylogeny, and its importance in pollination [119,120]. Hileman's [115] review demonstrates the repeated recruitment of the CYCLOIDEA (CYC) developmental programme for multiple transitions to bilateral symmetry (as well as reversals to radial symmetry), during the diversification of flowering plants. However, she also emphasizes that few of the specific underlying causes are known about the function or regulation of the CYC pathways, and that this knowledge is needed to understand how, and thus possibly why, there has been such frequent recruitment of the same pathway during diversification in floral symmetry.

Wessinger et al. [116] examine the genetic architecture of the transition from bee- to hummingbird-pollinated flowers in the genus Penstemon by studying the variation of characters in an F_2 hybrid population between two species having alternative pollination syndromes. This shift has occurred repeatedly in the genus as well as in other lineages [121]. Understanding the genetic basis of the suite of character changes involved in the transition between pollination syndromes provides insights into the tempo at which change could occur in adapting to another pollinating vector, which in turn could promote speciation [57]. The results of Wessinger et al. [116] indicate that the different components of the two floral syndromes are controlled by relatively few loci of medium to large phenotypic effect and that the traits are genetically linked. Another interesting result of their study is the failure to detect antagonistic pleiotropy, that is, they found no evidence that traits co-localizing to the same genomic regions include traits adaptive to alternative syndromes. In other words, all of the correlated traits are in the direction of adaptation to one syndrome. The lack of antagonistic pleiotropy would facilitate the transition between floral types. Taken in their totality, the results of Wessinger et al. [116] indicate that despite the pronounced differences between bee- and hummingbird-pollinated flowers, transitions, especially to the latter, may occur rapidly and repeatedly.

Future studies of floral transitions must be done within the framework of highly resolved, strongly supported phylogenies. Such phylogenetic resolution among species may be challenging in a group such as Penstemon [122], but it is necessary that bee to hummingbird floral transitions between sister species be studied for an accurate reconstruction of the genetic architecture of the transition as it occurred in nature. In addition, the phylogeny is essential for an accurate estimate of the number of parallel transitions in any clade and for understanding the ecological causes and consequences of the floral shifts.

Genome doubling or polyploidy was a major interest of Leslie Gottlieb. While polyploidy had long been of interest to plant evolutionists, from the early to well past the mid-twentieth century there was emphasis on documenting polyploidy by counting chromosomes and studying meiotic pairing in polyploids [123,124]. Among Gottlieb's many contributions to the study of polyploidy was his pioneering application of allozyme/isozyme data to the study of both recent and ancient polyploidy. He recognized that the genetic insights that could be achieved with isozyme data, though having limitations, went beyond what had been possible with other techniques, especially classic cytogenetics. He was the first to look at the genetic consequences of recent polyploidy [125], using as a model the recently formed polyploids in Tragopogon [126,127]. Realizing that the number of isozyme-coding genes was typically highly conserved in 'diploid' plants [128], he brought attention to the possibility of using isozyme gene number as a proxy for the investigation of older polyploidy events.

An intriguing question that Leslie Gottlieb posed concerned the degree to which gene expression differences observed among duplicate genes present in an allopolyploid might be a legacy of expression differences that were already present in the diploid parents of the polyploid [125,129]. He pointed out that the diploid parents of polyploids are not the same diploids as contemporary members of the same species. Rather, the diploids are members of the same lineage, and the more ancient

the polyploid event, the more tenuous the assumption of equating gene expression patterns in the parental and contemporary diploids. Gottlieb suggested that this problem could be alleviated to some extent by examining instances where polyploids are of very recent origin. These early papers by Gottlieb provide elegant demonstrations of how a relatively simple technique such as enzyme electrophoresis, combined with biochemical methods, could be used to address some basic issues in plant polyploidy such as gene expression patterns [129]. Buggs *et al.* [130] in this issue revisit this question of the legacy of the diploid parents in determining gene expression patterns in allopolyploids using, appropriately, much more extensive genomic data that now have become available for *Tragopogon.* After a review of this very active area of research using a variety of techniques, Buggs *et al.* [130] raise the important caution that parental legacy is typically not considered in modern genomics studies of allopolyploids.

Gottlieb's research on *Tragopogon* used isozyme data to show that an allopolyploid is 'far more than the sum of its genomic parts', to quote Soltis *et al.* [131, p. 2] in this issue. Again, using enzyme electrophoresis, Roose & Gottlieb [125] showed that tetraploid *Tragopogon* species not only have additive profiles but also contain novel forms of enzymes not present in their diploid parents. This is because the subunits encoded by alleles from different diploid parents can form novel functional multimeric enzymes, perhaps with different activities and with the potential for different functions. Roose & Gottlieb [125] recognized this and it is what intrigued them most about their results. Soltis *et al.* [131] enumerate the sources and potential consequences of evolutionary novelty in polyploids, which now can be investigated at the genomic scale with a variety of techniques. Given the combination of genomic shock (extreme mutability during the first generations after genome merger and duplication) and fractionation (potentially biased loss of genes or genomic segments during the ongoing process of diploidization), they raise the intriguing possibility that 'every individual in a polyploid population or species may be unique' (p. 9). Polyploidy as a powerful generator of genetic novelty thus has clear implications for ecological evolution.

The ecological causes and consequences of polyploidy are a long-standing theme in the history of polyploidy research, which Ramsey & Ramsey [132] review in this issue. The interest in ecology and polyploidy over many decades is not surprising given the documentation of the pervasiveness of polyploids in the green plant clade. Ramsey & Ramsey [132] discuss the different perspectives from which the ecology of polyploidy have been viewed and studied over time. They emphasize the challenges in disentangling the factors driving polyploidy and the diverse ecological attributes such as reproductive biology, microhabitat, phenology and geographical distribution that have been associated in various ways with genome doubling. These challenges include such basic issues as distinguishing cause and effect, and correlation versus causation. In the case of polyploidy, these involve determining whether traits seen in polyploids are the result of polyploidy *per se*, whether they function in the establishment and persistence of polyploids, or whether they are correlated with other traits not directly related to the environment of the polyploid.

Some of the leading plant evolutionists of the twentieth century were interested in ecology and polyploidy, but they brought particular perspectives to the topic, often reflections from their primary interests in cytogenetics and biosystematics [133,134]. Ramsey & Ramsey [132] trace the evolution and

broadening of the study of the ecology of polyploids as population biologists became interested in polyploidy [135–137] and as molecular techniques were applied to questions such as multiple origins of polyploids [138–140]. One of their most trenchant conclusions is that it is thus reasonable to hypothesize—though by no means proven—that polyploidy *per se* is a factor underlying ecological divergence of cytotype populations. They note that synthetically produced polyploid plants and the products of multiple origins represent particularly fruitful approaches for understanding the impact of polyploidy on ecology. The review by Ramsey & Ramsey [132] elegantly documents that any thorough study of the ecology of polyploids must, like most other issues in plant evolutionary biology, employ a multidisciplinary approach, a stance championed by Leslie Gottlieb throughout his career.

Neopolyploidy (recent polyploidy events) is easily detected in plant lineages where different ploidal levels can be documented with chromosome number determinations. However, the issue of whether the lowest number now seen in plant taxa are diploids or ancient polyploids (palaeopolyploids) has been discussed and debated for decades. One of the most widely employed criteria has been to specify critical numbers where values above a given value are considered polyploids and those below diploids [133,134,141]. That approach yielded estimates of polyploidy in extant flowering plants in the range of 35–50%, with the estimates for ferns much higher [133,141].

Leslie Gottlieb's landmark paper [128] reviewed the use of enzyme electrophoresis to assess ancient polyploidy. In that paper, he argued that, because the same catalytic reactions occur in different subcellular compartments, diploid plants have a minimal conserved number of loci for many of the enzymes assayed in electrophoretic studies. He reasoned that increases in the number of loci encoding enzymes catalysing a reaction (isozymes) above the minimal conserved number could occur in diploids where 'extra' isozymes would be expected at a few loci, or through polyploidy where increased isozyme number would be seen for many enzymes. However, even while arguing for the isozyme counting approach for inferring polyploidy, Gottlieb considered the situation in maize (corn) where he mused that although this important crop plant has a low chromosome number, the rather extensive duplications detected with the electrophoretic technique might be the result of more ancient polyploidy rather than duplications in a diploid genome [128]. As the results of electrophoretic studies by Gottlieb and others accumulated, it became apparent that duplications above the minimal conserved level were common in many presumably 'diploid' plants (using the chromosome number criterion), and it became increasingly difficult to classify plants as diploids or palaeopolyploids.

A definitive answer to the question of whether a plant was a diploid or an ancient polyploid that intrigued earlier workers, including Leslie Gottlieb, is now emerging. Genomic studies are providing documentation of the ubiquity of palaeopolyploidy in green plants [10,142,143]; as complete genome sequences are obtained for additional species, the same picture of extensive gene redundancy is revealed. Now that there is no question that there have been one or more rounds of polyploidy during green plant evolution, many questions about the timing and consequences of whole genome duplications are being addressed with genomic tools. The last two papers in this special issue by Vanneste *et al.* [144] and Jiao & Paterson [145]

provide an appreciation of the questions that can be addressed about the number, timing and potential consequences of ancient polyploidy events in the evolution of green plants.

Vanneste *et al*. [144] investigate the timing of ancient genome duplications in angiosperms for those plants that have genome sequencing data. Using modern dating methods, their results suggest that there was a burst of genome duplications at the end of the Cretaceous, corresponding closely with the Cretaceous–Tertiary mass extinction (approx. 66 Ma). Vanneste *et al*. [144] note the association between genome-wide duplication and environmental stress/fluctuation and speculate on possible reasons for these associations. They make the provocative suggestion that some of the key attributes of polyploids may have facilitated their successful establishment and diversification following that mass extinction, reshaping the modern landscape.

Jiao & Paterson [145] in this issue have examined gene content across the land plant tree of life looking for an association between genomic changes and morphological novelty. They reconstruct ancestral gene contents for key nodes of the land plant family tree. In their study, they report that widespread polyploidy in the flowering plants appears to be the major driver of diversification and morphological/physiological novelty. As stated by Jiao & Paterson [145], 'One of the great challenges of evolutionary genomics is linking genome modifications that are evident in the burgeoning sets of angiosperm (and other) genome sequences now available to speciation, diversification, and the morphological and/or physiological innovations that collectively constitute biodiversity'.

Taken collectively, the papers in this issue provide an appreciation of the variety of topics being investigated on polyploidy. The ages of polyploids being investigated range from 'new' synthetic polyploids to those that are the result of ancient genome-wide duplications. These polyploids of such differing ages facilitate the examination of the consequences of genome doubling from its inception through one or more duplications over millions of years of evolution. With the genomic tools now available, and a renewed appreciation of the value of ecological and population genetic studies, this is an exciting time for research on polyploidy and offers the potential for broader and deeper insights into the role it has played in plant evolution and in the generation of plant biodiversity. No doubt, Leslie Gottlieb would find this all very fascinating.

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