doi:10.20944/preprints202106.0494.v1

Review

Recent progress regarding the evolution and molecular aspect of insect gall formation

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Abstract: Galls are characteristic plant structures formed by hypertrophy (excessive increase in cell size) and/or hyperplasia (cell proliferation) induced by parasitic or pathogenic organisms. Insects are a major inducer of galls, and insect galls can occur on plant leaves, stems, floral buds, flowers, fruits, or roots. Many of these exhibit unique shapes, providing shelter and nutrients to the insects. To form unique gall structures, all-inducing insects are believed to secrete certain effector molecules and hijack host developmental programs. However, the molecular mechanisms of insect gall induction and development is still largely unknown because of the difficulty of studying non-model plants in the wild. Recent progress in next-generation sequencing has allowed us to determine the structure of biological processes in non-model organisms, including gall-inducing insects and their host plants. In this review, we first summarize the evolutionary aspects of gall-inducing life histories and their adaptive significance for insects and plants. Then, we briefly summarize recent progress regarding the molecular aspects of insect gall formation.

Keywords: adaptive significance; evolution of gall insects; gall-inducing insects; gall formation mechanism; insect effectors

1. Introduction

Galls are induced on plants by viruses, mycoplasma, bacteria, fungi, nematodes, insects, mites, and other plants and are accompanied by hypertrophy (overgrowth in size) and hyperplasia (cell proliferation) of plant tissue to generate a wide range of gall morphologies [1]. Galls induced by animals and plants are called zoocecidies and phytocecidies, respectively ('cecidie' means 'gall' in French; [2]). Among them, insect-induced galls have attracted the attention of many researchers because of their unique shapes and wide range of variation. It is estimated that more than 20,000 insect species can induce galls on plants (Fig. 1), and host plant species span numerous phylogenetic

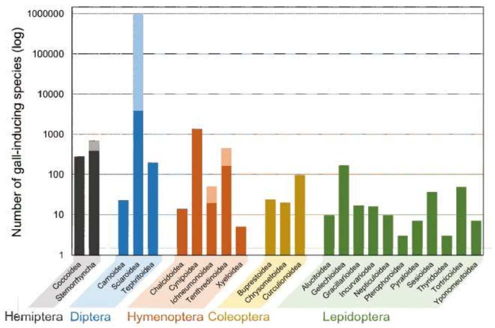

Figure 1. Number of gall-inducing insects species, grouped by super-families. Lighter colors depict highest estimates [25, 115, 116].

doi:10.20944/preprints202106.0494.v1

lineages, suggesting that gall-inducing systems have evolved independently during the evolution of insects [3-6].

Insect galls can be induced on plant leaves, stems, floral buds, flowers, fruits, or roots, and many exhibit unique shapes (Fig. 2).

These galls often look like fruits or floral buds, but their shapes are generally quite different from those that the respective plants originally bear. Therefore, it appears that gall-inducing insects hijack the plant developmental system to generate a novel organ in the plant. Gall-inducing insects produce stimuli that induce the initiation of the development and maintenance of gall tissue. Interestingly, phytohormones, such as indole acetic acid (IAA) and cytokinins, are detected at higher concentrations in gall-inducing insects rather than galls generated on host plants [7-11]. There is also evidence that certain amino acids and proteins are possible signals for gall induction [6]. These results suggest that substances transferred to plants via an insect ovipositor, secreted from larval saliva and/or excrement from larvae induce the reprogramming of plant cells [5,6,12].

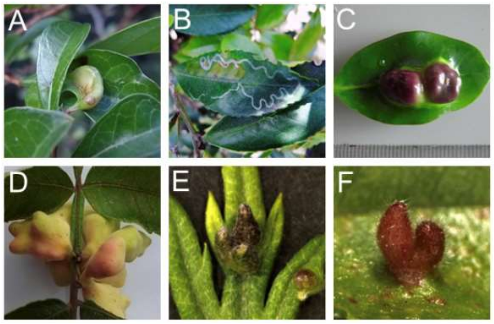

Figure 2. Examples of insect galls generated on leaves. (A) *Glochidion obovatum* gall by the micromoth *Caloptilia cecidophora*. (B) *Eurya japonica* gall by the micromoth *Borboryctis euryae*. (C) *Distylium recemosum* gall by the aphid *Neothoracaphis yanonis*. (D) *Rhus javanica* gall by the aphid *Schlechtendalia chinensis*. (E) *Artemisia montana* gall by the gall midge *Rhopalomyia yomogicola*. (F) *Ulmus parvifolia* gall by *Tetraneura akinire*. Panels A and D are from a previous study [114].

Plants generally have defense systems to protect them from predators, suggesting that gall-inducing insects overcome or repress plant defense responses. Several studies have shown that gall producers exploit their symbiont bacteria to produce effectors that disrupt plant defense [5]. Interestingly, insect-derived bruchins can stimulate neoplasm formation on host plant leaves, inhibiting larval entry into plants [13]. This could be a type of plant defense system that plants acquired to inhibit insect invasion.

The unique shapes and wide range of variation in galls have attracted the attention of researchers in entomology, botany, and ecology, as well as amateur nature enthusiasts. Each specific insect species generates a fixed gall shape on respective host plants, indicating that gall-inducing insects activate the specific developmental pathways of each host plant and tightly control these pathways. However, the molecular and cellular mechanisms of gall development are not yet well understood, and we need more studies on a variety of insect and plant taxa.

In this review, we introduce insect galls, especially regarding plant molecular biology, and hope to stimulate molecular plant studies of insect galls. We first review the evolutionary aspects of gall-inducing life histories of insects and plants and their adaptive significance. Then, we briefly review the recent progress in the molecular aspects of insect gall formation.

2. Evolution of gall formation in insects

Galls are presented by Darwin as examples of the great developmental plasticity of plants several times in the Origin of Species [14], showing that the morphology of living organisms can "[undergo] great modifications, independently of the gradual accumulation of slight inherited modifications." Subsequently, gall formation was the subject of a debate between supporters and opponents of Darwinian theory, the latter that the negative impact of galls on plants was in opposition to natural selection [15].

The low frequency of the gall-inducing lifestyle among herbivores, combined with the numerous and distant taxa bearing gall-inducing species, strongly suggests that the gall-inducing habit has appeared independently many times in insects. However, the evolutionary pathways leading to the acquisition of the ability to manipulate plant development remain unclear. The large diversity in gall morphology among gall-inducing insect species raises questions regarding the possible underlying mechanisms involved and the adaptive significance of this phenotype.

2.1. Pathways of gall evolution in insects

Based on Küster's classification of galls [16], Wells was the first to summarize the first time evolutionary hypotheses regarding the origin of galls [17] (Fig. 3A). Thanks to descriptions of gall development and with the help of the von Baer Laws [18], he suggested possible evolutionary pathways leading to the formation of galls and the evolution of their forms in each of the known orders containing gall-inducing insects. However, these scenarios were not supported by phylogeny and are now obsolete.

Progress in the phylogeny of gall-inducing insects and more detailed descriptions of the development of galls through the use of new technologies have provided more highly supported evolutionary pathways. Price described a two-pathway evolutionary scenario [3], wherein all gall-inducing insects evolved from an ancestral free-feeding herbivore, either through plant mining or through sedentary surface feeding (Fig. 3B).

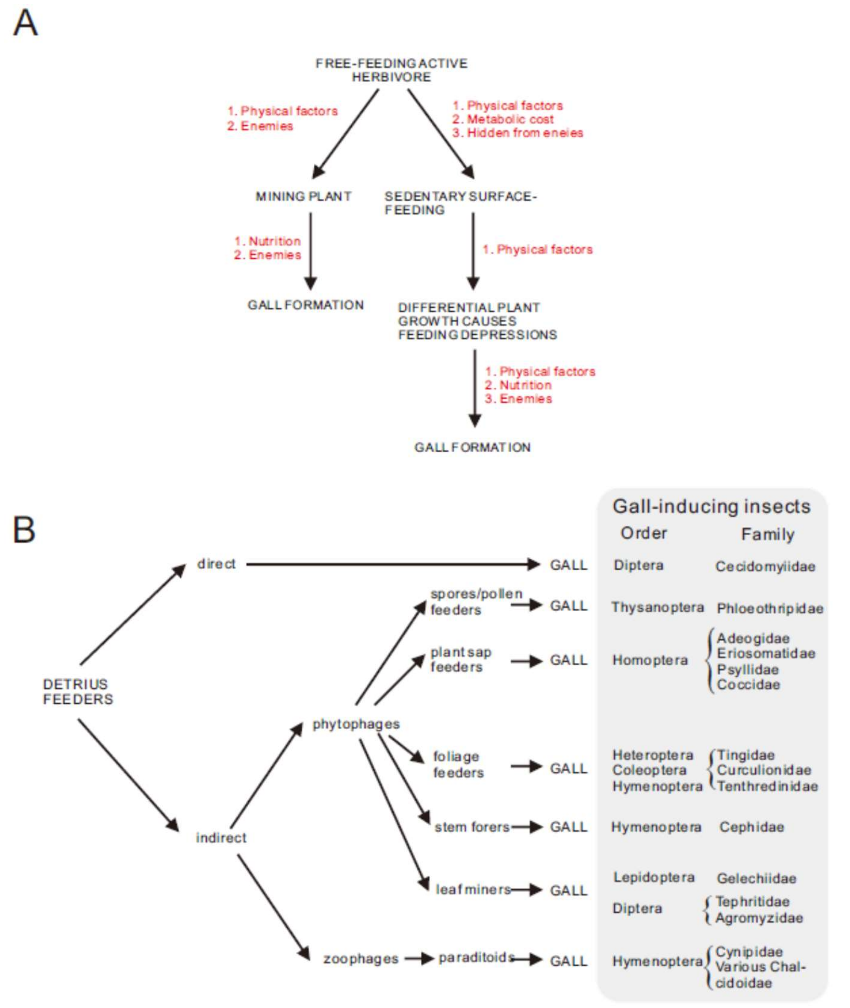

Figure 3. Evolutionary pathways proposed for gall induction. (A) The two routes of evolution of gall-induction from free-feeding active herbivores proposed by [3], with possible selective factors (in red). (B) Evolutionary pathways proposed by [117], with mention of insect families that may have followed them (on the right).

Two main objectives can be addressed in the Price scenario. First, the phylogeny of gall midges suggests that their ancestral lifestyle was that of fungus-feeders rather than plant feeders. Indeed, basal groups of Cecidomyiidae (Diptera) are mycetophagous, and some gall midges have retained an association with symbiotic fungi [19]. Moreover, several fungal genes that play a role in the interaction with the host plant are horizontally transferred into cecidomyids [20]. These results strongly suggest that mycetophagy is a preadaptation to gall induction in the cecidomyiids. Second, the Cynipidae family (Hymenoptera), another large group of gall-inducers, is excluded from the scenario because their ancestral state was unlikely a surface-feeder or plant-miner based on their phylogeny [21].

A more complete hypothesis has recently been proposed for the evolutionary pathway of gall-inducers [19] (Fig. 3C). Under this scenario, gall-inducing abilities evolved from plant mining (leaf-miners and stem-borers), ectophagous lifestyles (foliage feeders, sap-feeders, and pollen-feeders), fungus-feeders, or parasitoids. The parasitoid pathway received strong support from phylogenetic analyses in the case of Cynipidae [21], Chalcidoidea [22], and Braconidae [23] (Hymenoptera). The origin of foliage feeders in

doi:10.20944/preprints202106.0494.v1

Tenthredinae (Hymenoptera) was also confirmed by phylogeny [24]. In contrast, the origin of leaf-miners has rarely been reported. Most gall-inducing Gelechiidae (Lepidoptera) make stem galls, suggesting an origin from stem-borers [25]. Gall-inducing fruit flies may have evolved from ovary miners [26]. Based on phylogeny, the origin of gall induction from leaf-miners is likely for the *Mompha* genus [27] (Lepidoptera). Agromyzidae (Diptera) mostly consists of leaf-miners, and gall-inducing species are rare in this family, making leaf-miners a likely ancestral state [28]. A similar hypothesis can be drawn for Gracillariidae (Lepidoptera), which mostly consists of leaf-miners.

3. Adaptive significance of galls for insects

Three main hypotheses have been proposed regarding the adaptive value of insect galls [6]: (i) the nutrition, (ii) natural-enemy, and (iii) microenvironment hypothesis.

3.1. Nutritive hypothesis

In its simplest form, the nutrition hypothesis states that feeding on gall tissues provides enhanced nutrition compared to other available plant tissues, resulting in the higher performance of the gall-inducer compared to that of other feeding guilds [3]. The broader definition includes examples where plant manipulation does not lead to an apparent increase in nutrients but rather a control of the nutrient levels in galls to match the nutritional requirements of gall-inducing insects [29].

This is an adaptive hypothesis that has received the most experimental support through four main approaches: (i) histochemical, biochemical, or metabolomic analyses of gall nutrient content compared to ungalled tissues; (ii) experimental manipulation of host plant nutrition to determine whether the gall-inducer controls gall nutrient levels; (iii) investigation of the fitness consequences for gall-inducers of gall nutrient level manipulation; and (iv) performance of non-gall-inducing insects when feeding on galls.

There is a considerable amount of research showing the enhanced nutritional quality of galls [5]. In many gall-inducing insects, the insect-feeding site is turned into mobilizing sinks that accumulate nutrients, making them available to the larvae. Among these, gall-inducing aphids have been the most studied taxa. The use of carbon-14 labeling revealed that gall-inducing aphids cause an accumulation of carbon compounds, most likely sugars, in the gall tissues [30, 31]. Gall midges manipulate the carbon:nitrogen ratio (C:N), producing various trends according to the species. *Daphnephila taiwanensis* and *Daphnephila sueyenae* on *Machilus thunbergii* increased the C:N ratio in the gall [32], whereas the attack of Hessian flies on wheat [33] and infestation of Asian rice gall midges on rice [34] decreased the C:N ratio. Gall wasps have also been found to induce carbohydrate accumulation in gall tissues [35-37]. For organisms feeding on plants, enhanced nutrition may also imply control of plant defenses, and both the primary and secondary metabolism of plants can be affected [38]. Cynipids and sawflies have been found to improve their food source quality by decreasing the levels of defensive compounds in the gall compared to other plant tissues (tannins and phenols) [39, 40].

However, high nutrient levels do not always benefit insects, and the accumulation of primary metabolites is not sufficient to demonstrate the nutritive hypothesis [29, 41]. The first criterion is that the gall-inducer must control the nutrient levels within the gall. This was demonstrated in gall-inducing aphids, which maintain the nutritional quality of their feeding site even when plant nutrition is modulated by the addition of synthetic fertilizers [42, 43]. Similarly, the nutritional environment of the thistle gall fly, *Urophora cardui*, remains stable and favorable to the insect despite alterations in plant nutrition [44]. Nutritional homeostasis to match the nutritional requirements of insects is key to the strategy developed by plant manipulators [45]. The second line of evidence in favor of the nutrition

doi:10.20944/preprints202106.0494.v1

hypothesis is the better performance of gall-inducers on gall tissues. An increase in insect survivorship was first detected in cynipids, where artificially enhanced nitrogen levels in galls impaired insect survivorship [31]. Similar manipulation of gall nutritional status also decreased thistle gall fly performance [45]. Among the gall-inducing aphids, *Tetraneura* species associated with gall tissues with higher concentrations of total amino acids produced more offspring [46]. However, measurements of insect performance on galls may confound the adaptation to galls with the increased nutritional value of galls [47]. Following this criticism, the high performance of non-gall-inducing insects on gall tissues would be a stronger argument in favor of the nutrition hypothesis. Several free-living aphids belonging to non-gall-inducing species perform better on aphid galls [48–50]. The endophagous caterpillar of the *Hellinsia glenni* moth grew better and had a higher emergence rate when transplanted from the stem to gall tissues [51]. Finally, opportunistic beetles perform better on galls than on other plant tissues [52].

3.2. Enemy hypothesis

The enemy hypothesis states that the gall protects the insect from its natural enemies and pathogens. Most galls are enclosed environments, and attacks must cross the gall tissues. Therefore, morphological features that make it difficult to reach the gall-inducer should be adaptive. Confirming this hypothesis would ideally require a comparison of the mortality of a gall-inducer with its non-galling ancestor, which is impossible for long-established gall-inducer lineages [6]. Because direct evidence for the enemy hypothesis is impossible to obtain, studies have examined indirect support by comparing the mortality rate of gall-inducing and non-gall-inducing species and detecting correlations between morphological traits of galls and changes in insect mortality rates.

Comparison of the mortality of gall-inducers with those of other feeding guilds has been assessed in only a few cases. Gall-inducing sawflies are attacked by fewer parasitoid species and experience lower mortality rates than free-living forms [53]. This effect has also been tested in tortricid moths, among which gall-inducers are rare, and galls appeared to provide better protection against parasitoid colonization than any other larval feeding habit [54]. Gall induction also affects the shape of the enemy communities. The gall-inducing lifestyle excluded some parasitoid groups among the sawflies, especially those lacking ovipositors, such as tachinids [53]. However, these results cannot be generalized to all gall-inducing insects. Although concealment of endophagous insects provides them with better protection against enemies compared to ectophagous species [54], differences in mortality because of natural enemies between gall-inducing and non-gall-inducing endophagous insects is almost equivocal. Meta-analyses of mortality across herbivorous feeding habits show that gall-inducers tend to be better protected against parasitoids than leaf-miners [55], whereas others indicate that the survival rates of leaf-miners and gall-inducers are similar [56]. Other analyses suggest that parasitoid richness in gall-inducers is equivalent to ectophagous insects and higher than in other endophagous groups [57, 58]. Therefore, the adaptive value of gall protection against enemies is probably not supported as a long-term hypothesis.

The fact that galls do not represent an efficient defense against enemies, in general, can result from counter-adaptations by enemies. Identifying such counter-adaptations in natural enemies provides indirect evidence for the enemy hypothesis. Interestingly, the mortality curves of herbivorous insects can also provide indirect evidence for the enemy hypothesis. Although the leaf-miner survival rate decreases linearly during its development, the mortality of gall-inducers shows a peak at earlier stages of development [56, 59]. This "window of vulnerability" in the earlier steps of insect development is presumably caused by undeveloped gall structures and therefore supports the protective value of galls.

Gall-forming herbivores exhibit considerable variations in gall morphology, and this diversity is ideal for testing whether gall shapes affect enemy attacks. Many studies have tested the "Gall diameter hypothesis," which posits that larval accessibility for natural enemies is controlled by gall sizes [60]. For example, cynipid larvae found in the gall are the least attacked by parasitoids [61]. In *Eurytoma gigantea*, insects that induce larger galls are generally favored by natural selection [62]. *Urophora cardui* survival against parasitoids also increases with the number of larval chambers and then gall size [63]. More recently, a negative correlation between parasitoid attack rates and gall sizes was demonstrated in *Diplolepis rodase* [64], supporting the gall diameter hypothesis. However, the same authors found that a larger gall size makes them more vulnerable to bird attacks [65]. Additionally, studies on gall midges did not support the gall diameter hypothesis [66, 67], suggesting that other gall traits should be considered, such as toughness, hairiness, and stickiness, which could negatively correlate with parasitism rates [68]. Additional traits can also provide indirect defense against enemies. The surface of some cynipid galls is covered by extrafloral nectaries, encouraging the recruitment of ant guards [69, 70, 71]. Gall-inducers can also affect volatile plant emissions, such as reducing volatile organic compounds used by parasitoids to localize gall-induced plants. This has been demonstrated in the gall-inducing aphid *Tetraneura nigriabdominalis* [72], *Eurosta solidaginis* [73], and psyllid galls [74]. Gall-inducers, such as the aphid *Slavum wertheimae,* can also induce the emission of odors that repel browsing mammals [75].

Finally, shapes and colors have been proposed to be a form of aposematism, meaning that the external appearance of the gall prevents herbivores from damaging the gall [76, 77]. However, the outer surface of some galls turns red because of the accumulation of secondary metabolites, such as anthocyanins (Fig. 2). Red is the contrast color of green and must be remarkable to birds or mammalian animals, a candidate predator of insects. Such coloration may be caused by early senescence of the plant tissue for nutrient translocation and/or an adjunct during gall induction caused by cytokinin and sugar [76, 78, 79]. Accumulation of anthocyanin could be a plant response to removing the galls by predators, such as birds, but evidence supporting this hypothesis has not yet been reported. Alternatively, because galls carry molecular features, such as flowers and fruits (shown by transcriptome analyses, see below), the coloration of galls is one of the characteristics of these reproductive organs. There are several other hypotheses, such that the coloration of galls would be an interesting phenomenon worth investigating regarding adaptive significance.

3.3. Microenvironment hypothesis

According to the microenvironment hypothesis, or the harsh environment hypothesis, the gall provides better physical conditions than the external environment [3]. This adaptive hypothesis has been suggested because, on a global scale, the fauna of gall-inducing insects is richer in areas where hygrothermal stress is higher [80]. Gall-inducing insects are more abundant at a regional scale and survive better in xeric habitats than in mesic habitats [81]. Similarly, the dry and hot environment of the Amazonian upper canopy host has among one of the highest diversities of gall-inducing insects [82]. However, this trend is not always clear and may vary among regions. For instance, Ribeiro and Basset [83] also found positive gall establishment and survivorship in the upper canopy in Australia; however, the authors insisted that this diversity relied most on a low number of "super-host" plants. In Australia, no correlation occurred between the number of gall-inducing species and rainfall [84]. Interestingly, this study identified an increasing trend in the number of gall-inducing species per plant based on the decrease in rainfall, suggesting that dryness favors the occurrence of "super-hosts" [84]. Finally, the study of South African fauna did not reveal any link between hygrothermal stress and gall abundance [85].

doi:10.20944/preprints202106.0494.v1

Some studies tested this hypothesis at the species level. For instance, the gall midge *Mikiola fagi* was more abundant at forest edges, suggesting a preference for a dryer environment [86]. Fay et al. [36] proposed that the manipulation of water fluxes in the plant by the cynips *Antistrophus silphii* may control humidity within the gall, which could protect against stress during drought. The most convincing evidence of the microenvironment hypothesis was obtained for the cynips *Andricus quercuscalifornicus*, which maintains humidity levels near saturation within the gall chamber, resulting in a positive effect on larval survival [87]. Behavioral studies have also shown indirect support for this hypothesis. When a hole is made on the closed gall of *Distylium racemosum* generated by the social aphid *Nipponaphis monzeni*, many soldier nymphs inside the gall gather at the hole and discharge their body fluid to plug it and activate plant cell proliferation around the hole [88]. This also suggests that the gallers actively maintain the microenvironment within the gall, but this repair behavior is also important in preventing further invasion of enemies or other herbivores.

However, gall morphology occasionally misleadingly suggests a high level of protection from environmental conditions. For example, the expected greater resistance to desiccation for galls on *Metrosideros polymorpha* because of the abundant trichomes encountered on their surface has been contradicted by experimentation [89].

4. Benefits for plants

Galls do not appear to carry adaptive benefits for plants; on the contrary, they negatively affect plant growth or have seemingly no effect in many cases. Plants may generate galls to localize the parasitic galler insects in a limited space, such that plants can inhibit the spread of damage [1]. However, evidence to support this hypothesis is very poor [3]. Another hypothesis is that plants use excrement from galling insects by absorbing them from the galls. In closed galls of *Distylium racemosum* and *Syrax japonicus*, honeydew from gall-inducing aphids is absorbed by the galls through the plant vascular system, and this mechanism helps aphids avoid drowning in their secretions within the gall. This suggests the possibility that plants use the secretions from gall-inducing insects; however, the sugar concentration of honeydew from these aphids is low and devoid of sucrose [90]. Another example showed that *Eucalyptus* plants with galls exhibit indirect effects resulting in tolerance to cold injury, compared to those without galls [91]. Although these examples imply some benefits for plants from galls, direct or indirect evidence is required to elucidate the plant benefits derived from galls.

5. Molecular biology of insect gall formation

5.1. Changes in plant hormonal regulation during gall development

Phytohormones produced by gall-inducing insects have long been hypothesized to play a key role in inducing gall structures to exogenously control phytohormone regulatory pathways in host plants [8, 11, 92, 93, 94, 95]. Indeed, a transcriptome analysis of psyllid galls on the Hawaiian *Metrosideros polymorpha* showed that auxin response genes were upregulated in galls [96]. In *Rhus chinensis* and *Rhus javanica* gall formation, the genes involved in auxin and abscisic acid (ABA) metabolic and signal transduction pathways are significantly activated [97, 98].

5.2. Changes in the expression patterns of genes involved in the biosynthesis of the metabolic process during gall formation

Tannins are crucial in the protection of host plants and gall-inducing insects from herbivory. Aphis galls on *Rhus chinensis* accumulate gallotannin, and genes involved in gallotannin biosynthesis [99], gallic acid synthesis [98], and lignin biosynthesis [97] have been identified. In the developing gall of the chestnut gall wasp, *Dryocosmus kuriphilus*, on the Chinese chestnut, *Castanea mollissima*, the expression of genes related to metabolic processes, such as phenylpropanoid biosynthesis, secondary metabolism, and plant-pathogen interactions, was altered compared to that of non-infested leaves [100]. Galls induced on elm leaves by a gall-inducing aphid, *Tetraneura akinire*, were shown to express the genes encoding lignocellulose synthase, suggesting the reinforcement of cell walls to improve resistance to damage by the aphid [101].

5.3. Candidate insect effectors for gall formation

The common characteristics of complex insect gall structures include the lignified outside cell layer, which has a protective function against natural enemies and the outside environment, and the nutritive tissues of callus-like cells, which contain insect nutrients and vascular tissues for transporting nutrients to these tissues [1, 6, 102, 103]. To construct gall structures, many gall-inducing insects are believed to have the ability to secrete certain effectors, including plant hormones, into plant tissues using their mouthparts or ovipositors to induce gall formation in the host plants [5, 104, 105]. To induce complex gall structures in plant tissues, gall-inducing insects must control the cellular machinery of their hosts, including development, metabolism, chemistry, and physiology, and hijack host gene expression programs in favor of those of the insect. However, little is known about the gene expression profiles in developing galls because of the difficulty of studying non-model plants in the wild. Recent progress in next-generation sequencing (NGS) has allowed us to determine the structure of biological processes in non-model organisms, including gall-inducing insects and their host plants. Several transcriptome studies have identified candidate gall-inducing effectors from insects [106 -108]. Recently, a novel insect secretory protein, designated as BICYCLE, was identified from the gall-inducing aphid *Hormaphis cornu*, which produces distinctive cone galls on the leaves of *Hamamelis virginiana* [109]. Bicycle genes are most strongly expressed in the salivary glands of gall-inducing generations. These results suggest that BICYCLE proteins regulate the gall development process in *Hamamelis virginiana*. Thus, several effector candidates were identified. However, because of the lack of model systems for analyzing gall-inducing insect and host plant interactions, no one has demonstrated whether these effector candidates indeed have a gall-inducing function. Future studies are needed to establish a model system for assessing gall induction and development processes at the molecular level.

5.4. Regulation of transcriptional factors for reproductive organ development

Darwin (1868) proposed that the shapes of complex insect galls resemble flowers or fruits [14]. Indeed, many remarkable flower- and fruit-like structures have been observed in insect galls, in particular those induced by gall midges, aphids, and cynipids in various host plant species [110], suggesting that the formation of gall tissues is similar to the development of flowers or fruits [111, 112]. It has recently been shown that genes involved in the development of flowers and fruits are activated in the leaf galls in *Vitis riparia* induced by the phylloxera, *Daktulosphaira vitifoliae* [112].

Activation of reproductive organ genes has also been reported during the early development of galls on *Rhus javanica* induced by a gall-inducing aphid, *Schlechtendalia chinensis*. Additionally, class-1 KNOX transcription factors were shown to be overexpressed ectopically in the early developmental gall tissue of *Rhus javanica*. Because the class-1 KNOX transcription factors are known to lead to the formation of *de novo* meristematic structures in leaves [113], the results support the hypothesis that gall-inducing insects convert source tissues into meristematic tissues by expressing the class-1 KNOX genes in leaf tissue cells. Collectively, we propose the following molecular mechanisms in the early stage of gall formation: (i) an ectopic meristematic structure is generated by the overexpression of class-1 KNOX genes, (ii) ectopic meristem is converted into a floral-like meristem by the expression of LFY, (iii) floral-like meristem develops to form fruit-like gall structures induced by the expression of floral regulatory genes, and (iv) photosynthetic genes are downregulated, whereas transporter and secondary metabolic genes are upregulated to alter tissue functions during the conversion of tissue function from source to sink organs [97].

This hypothesis was improved by comparative transcriptome analysis using four different insect galls generated on leaves: galls on *Glochidion obovatum* induced by the micromoth *Caloptilia cecidophora*, on *Eurya japonica* by the micromoth *Borboryctis euryae*, *Rhus javanica* by the aphid *Schlechtendalia chinensis*, and on *Artemisia montana* by the gall midge *Rhopalomyia yomogicola*. Comparison of these different galls revealed that (1) photosynthetic genes are downregulated in galls, supporting the hypothesis that galls are converted to sink organs rather than source organs on leaves, (2) developmental, cell cycle, and phytohormone genes are upregulated in galls, and (3) approximately 40 genes are commonly upregulated in these four galls. In particular, several key regulators of floral organ development, including SEPALLATA, AGAMOUS, and APETALA1, were commonly upregulated in *Artemisia montana, Glochidion obobatum*, and *Rhus javanica* galls [114]. Intriguingly, these floral organ regulatory transcription factors were not expressed in the *Eurya japonica* gall tissues. This gall has rather thin layers with a simple pouch-type gall structure compared with the other fruit-type galls, suggesting that these transcription factors do not induce the galls of *Eurya japonica*. We realize that the comparison of only four species is not sufficient to cover the wide range of variation in gall morphology. However, the accumulation of this kind of comparable data will reveal the common mechanisms of gall development and also the ones that generate diversity in galls [114].

6. Conclusion

Herein, we review the evolutionary pathways of the gall-inducing lifestyle in insects, its adaptive significance, and molecular aspects of insect gall induction and development. The ancestral lifestyle of gall-inducing insects varies among taxa, from ectophagous herbivores (foliage feeders, sap-feeders, and pollen-feeders), plant-mining herbivores (leaf-miners and stem-borers), and fungus-feeders to parasitoids. The origin of leaf-miners has rarely been reported. Regarding the adaptive significance of galls for insects, the nutritive hypothesis has received the most experimental support through various direct approaches, including chemical analyses and bioassays. In contrast, enemy and microenvironment hypotheses are only supported by indirect evidence, such as comparisons of predation rates by enemies between taxa with and without a gall-inducing lifestyle and comparisons of the numbers of gall-inducing species between xeric and mesic habitats. Although gall morphologies have received much attention in terms of protection from predators and harsh environments, we note that few studies have been conducted on the coloration of galls. The adaptive significance of coloration would be an interesting topic worth investigating. Very few studies have been conducted on plant benefits of galls, but there is an example that indicates that gall-induced plants have increase tolerance; thus, more studies are needed to elucidate the generality of this phenomenon. Finally, we note that an increasing number of studies challenge the molecular aspect of gall induction and development, and recent studies have revealed that the formation of gall tissues is similar to the development of flowers or fruits in terms of gene expression patterns. Therefore, studies using a wide range of gall-inducing insects and their host plant taxa should yield more detailed insights into molecular-level insect-plant interactions, which may be divided into subcategories. These studies should provide concise and precise descriptions of the experimental results, their interpretation, and the experimental conclusions drawn.

Author Contributions: M.H.S, S.T., and I.S. conceived and planned the manuscript. A. G., S. T, T. H., I. O. and M.H.S. wrote the manuscript.

Funding: This work was supported by JSPS KAKENHI Grant Numbers JP21K06234 to S.T., 19H00933 to M.H. S., and 17H06260 to I.O.

Acknowledgments: We would like to thank Editage (www.editage.com) for English language editing.

Conflicts of Interest: The authors declare no conflict of interest.

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