

Discovery of the *bicycle* gene family provides new insights into insect manipulation of plant development during gall induction

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EVALUATION OF



A novel family of secreted insect proteins linked to plant gall development

Korgaonkar *et al.*

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Galls are complex structures that develop from plant tissue, providing protection and food for gall-forming organisms, such as insects or mites. However, the molecules used by insects or mites to manipulate plant development have proved elusive. A landmark study has tracked down a gene in a gall-forming aphid that controls whether galls on witch hazel are green or red. The ‘green allele’ is strongly expressed in aphid salivary glands and represses plant genes used for red color formation. Excitingly, the gene product is part of a large suite of proteins that aphids may use to interact with plant biology.

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Background

When we think about the genetic control of growth and development in plants or animals, we naturally turn to the genome of the organism that is developing. But the development of one species can sometimes be controlled by the genes of a different species. Galls present an interesting example. Galls are unusual plant tissues whose development is induced by an interacting organism. Well-known galls include the root nodules of legumes induced by *Rhizobium* bacteria. The most complex galls are induced by animals, including certain nematodes, flies, wasps, aphids, and mites, for which the gall structure provides protection and food for the larvae (and sometimes adults) of the gall-inducing species. Importantly, galls are not amorphous growths but are intricately patterned and reproducible structures with a shape, color, and form specific to the animal, not specific to the host plant (one plant species can develop distinct gall structures induced by different animals). The assumption is that galls represent what Richard Dawkins has termed the ‘extended phenotypes’ of gall-inducer genes acting in host plant tissues^{1,2}.

It has long been clear that insects and mites must use molecules to manipulate plant cell biology to drive the development of galls, but, until now, the molecules responsible, and the genes encoding them, have not been identified. Many candidate genes have been proposed, based on transcriptomic and proteomic screens in gall-inducing animals, but linking particular animal genes to specific plant phenotypes has proved elusive³⁻¹¹. The genomes of a growing number of insects, including gall inducers, have been shown to encode genes for synthesis of plant hormones, including auxins and cytokinins, but how the products of these genes interact with plant tissues remains little understood¹². The report

from Korgaonkar and colleagues¹³ makes a leap forward in our understanding by identifying an insect gene that controls the color of galls formed on the leaves of the witch hazel shrub. Notably, the gene is a member of a novel gene family that may encode a suite of secreted proteins that are transferred from the insect into the leaf to manipulate plant development.

Main contributions and importance

A powerful approach to finding genes responsible for any phenotype is to exploit naturally occurring polymorphisms within a species. Starting with this logic, the researchers from the laboratory of David Stern observed that ‘cone galls’ made by the aphid *Hormaphis cornu* on leaves of witch hazel *Hamelis virginiana* are either red or green (Figure 1). These galls are formed by the proliferation of plant cells at the site where female aphids founding a gall insert their piercing mouthparts, suggesting that the red or green galls are induced by chemicals in aphid saliva. The investigators sequenced and assembled a reference genome of the aphid *H. cornu*, then undertook lower coverage genome sequencing of 43 aphids that founded green galls and 47 aphids that founded red galls. A genome-wide association study (GWAS) revealed several polymorphisms around a novel gene that correlated strongly with the color difference; this association was strengthened by the genetic analysis of hundreds more samples¹³. The clear implication is that this aphid gene controls the color of the developing plant structure.

The researchers named the novel gene *determinant of gall color (dgc)*. Green galls are far more common than red galls. Consistent with this, a closer analysis of polymorphisms indicated that the ‘green’ allele is ancestral in this aphid species; furthermore, the ‘red’ allele is dominant to the green. Working



Figure 1. Red and green galls induced by *Hormaphis cornus* aphids on leaves of witch hazel *Hamamelis virginiana* at Janelia Research Campus, Ashburn, Virginia

This image was reproduced with permission from David L. Stern.

out how the aphid *dgc* gene controls plant gall color is not straightforward. The first clue comes from gene expression. The common ‘green’ allele of the *dgc* gene was found to be expressed in salivary glands, specifically in the generation and life cycle stage that induces galls. (The derived ‘red’ allele showed low expression and repressed the ‘green’ allele.) The second clue comes from gene expression changes in the plant when a gall is induced. A striking finding is that high levels of *dgc* gene expression in the aphid repress transcription of plant genes encoding enzymes in the biosynthesis pathway for anthocyanins — compounds that include the red pigments found in many insect-induced galls^{14,15}; conversely, low *dgc* expression permits abundant anthocyanin production and a red pigment is formed. These findings suggest that *dgc* encodes a secreted protein that is injected into the plant to suppress the anthocyanin biosynthesis pathway.

There is more to a gall than its color. The aphid *dgc* gene seems to influence color, so how could all the other features of galls be induced, such as the altered rates and planes of cell division that must be necessary to form a cone gall? This study does not address these questions directly, but it provides some

tantalizing clues. Transcriptomic analysis of salivary glands, comparing gall-forming and non-gall-forming phases of the life cycle, revealed a large number of novel aphid genes putatively encoding secreted proteins specific to gall-inducing stages. Many of these genes share similar sequence characteristics, indicative of the expansion of a gene family by gene duplication. Indeed, over 400 genes encode predicted proteins with a common structure: a diagnostic pair of cysteine-tyrosine-cysteine (CYC) motifs that prompted the authors to give these the appealing name of *bicycle* (*bi-CYC-like*) genes. The color determinant gene *dgc* is one of these genes. Furthermore, the *bicycle* genes show hallmarks of having been subject to positive Darwinian selection, compatible with adaptation or even an evolutionary arms race with plants. The suggestion is that *bicycle* genes encode a vast suite of proteins that are injected into host plants to orchestrate gall formation.

Open questions

The strategy used for tracking down the *dgc* gene was essentially a GWAS approach. GWAS has its pitfalls, and in some research areas, it is not always clear whether a genetic polymorphism correlating with

a phenotypic trait is a causative variant or a spurious association, perhaps due to underlying genetic structure in the samples compared^{16,17}. In our view, this is unlikely to be a problem in the current study. The GWAS analyses in this study are carefully controlled, and the subsequent, very extensive, follow-on work summarized above is internally consistent with the association proposed. The link between *dgc* and gall color seems sound.

It could also be argued that rigorous proof showing that *dgc* controls gall color requires a direct manipulative experiment. For example, since the ‘green’ allele has far higher expression than the ‘red’ allele, it might be possible to use CRISPR/Cas9 or RNA interference (RNAi) approaches to disrupt *dgc* gene expression in aphids homozygous for the ‘green’ allele, and then test whether the gene-targeted aphids now induce red galls. A more straightforward alternative would be to compare the effect of the ‘red’ or ‘green’ allele in transient expression assays, for example in *Nicotiana benthamiana* leaves¹⁸.

What is not addressed in the study is whether there is any adaptive significance of differences between green and red gall phenotypes for the gall inducer^{14,15}. Another question that remains puzzling is how the secreted *dgc* protein controls the expression of plant genes. Does *dgc* encode a transcription factor or a co-factor that interacts with plant transcription factors? Does it influence transcriptional pathways more indirectly? Given that the expression of eight

genes in the anthocyanin pathway is suppressed, it is likely that the *dgc* protein targets a regulator of the pathway, possibly the MYB-bHLH complex¹⁹. Although the impact on the transcription of genes encoding this complex would be easy to assess, biochemical work would be necessary to identify perturbations to protein function.

But perhaps the most tantalizing questions concern the other *bicycle* genes, of which there are hundreds. Are all of these involved in aspects of gall formation? This seems unlikely since subsequent work has identified some *bicycle* genes in non-gall-forming aphids²⁰. Another possibility is that *bicycle* genes, and other secreted salivary molecules, have long been used by insects to subtly interact with plant biochemistry during feeding, and then some of these genes were later co-opted for evolutionarily new roles in gall formation. These will be fascinating questions to address.

Conclusion

The *dgc* gene identified by the authors is part of a large multigene family of putatively secreted proteins in the salivary glands of aphids and is a clear example of an insect gene that controls an aspect of plant development. A hypothesis emerges that there are a huge number of similar signaling molecules that aphids inject into plants that then change all kinds of plant cellular behaviors. This study could be a major step forward in the field.

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