

### Three person views of the future of gall research.

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We three have been working together on the biology of galling insects for 20 years – a scary thought, and not one that we all planned. Graham completed his PhD on solitary bees in 1990 and found himself perfectly qualified for a field in which, according to an expert, everything had now been done. Brilliant! Then came a call for someone to join a minibus trip to Bulgaria in search of the origin of something bizarre called a Knopper (see plate 1). Karsten and I met then (Karsten already having worked on spangle galls), both working at Silwood Park. A few years later, letters we posted to many botanic gardens and institutes in Hungary found their way to György (Gyuri) Csoka, an expert in forest protection, gall enthusiast and nature photographer, and our team was made. While we have grown older, and now our children hunt for galls too, the scientific questions that make galls fascinating remain very much the same, and still general enough that we can get government funding to work on them. The same is true for a small number of other scientific research groups around the world. Our focus has been gallwasps, but others work on the same general questions in other insects, nematodes, bacteria and fungi. Every few years this community comes together for a conference on the biology of gall-inducing arthropods, most recently in Brazil.

Very broadly, the questions seen as most important in gall research are the following: How do they do it? How did galling evolve? Why do galls come in such amazing shapes and sizes? And how can I tell all the critters in a gall apart?

**How do they do it?** In many ways this is the most exciting and challenging question of all. Something the gall-inducer produces causes a fundamental change in the way plant tissues develop, sometimes even reversing an ageing process to produce young tissues that can grow into something new. In some cases the process of gall induction is more or less understood – for some gall-inducing bacteria such as those in legume root nodules, and for a really serious nematode pest of root vegetables called *Meloidogyne*. Scientists in the USA are chipping away at gall induction in another pest – the hessian fly *Myetiola destructor*, which is a gallmidge pest of wheat – but the process remains little understood. Even less is known about induction of the structurally complex galls inhabited by gallwasps, other gall midges (for example, the *Asphondylia sarothamni* shown in plate 2), aphids, sawflies and others. We don't know if the mechanisms used by these different groups tap into the same plant developmental processes, or target quite different ones. The alternative mechanisms more or less remain those proposed by Howard Cornell in an 'ideas' research paper published in 1983 on the 'Why and How' of gall induction; either the parents inject something into the host plant when they lay their eggs, or the immature stages inject something as they feed. What is injected must either be the stimulus itself (such as a chemical that acts as a plant hormone), or something that gives rise to the stimulus after it has spread into plant tissues (such as genetic material – DNA or RNA - from the gallwasp or an accompanying virus or bacterium). Insects make widespread use of viruses in parasitic lifestyles – for example, some parasitic wasps inject viruses into other insects when they attack them, and the viruses overcome the immune system of the victims. Sometimes it is even more complex than this – the virus particles are not independent of the insect, but are coded for by the insect's own DNA. The insect controls

the manufacture of the virus particles and stocks them not with viral genes, but its own DNA. The viral particles are just a convenient way of exporting DNA outside the parasite's body. Gallwasps and other gall-inducers in the Hymenoptera have this system, and may well use it to hijack plant development.

When Howard Cornell wrote his paper, technology did not allow detection of which genes were 'switched on' in the gall inducer and in the plant during crucial stages of the gall-building process. Now this can be done. In Edinburgh we are looking at which genes are active right at the beginning of gall induction by the oak apple gallwasp, *Biorhiza pallida*. In gallwasps, gall growth needs a living larva (ie it isn't controlled just by the egg laying mother wasp), and most of gall induction happens when this larva is very small. We are comparing the gene activation patterns of very young larvae and older larvae. The idea is that genes that are much more active in young larvae than older larvae are our candidates for gall induction. We know that gallwasps have virus particle genes in their DNA, but working out whether these play any role in delivering the gall induction stimulus is another can of worms entirely! Our challenge as gall induction researchers is to attract enough research funding to chip away at this fascinating problem with new technology – and this is not at all easy!

**How did galling evolve?** Galling has evolved in such a diversity of organisms that there probably is no single underlying answer to this question. Gall inducing insects have evolved in groups that are non-galling herbivores (such as aphids and beetles) and in groups of parasites that attack herbivores hidden inside plants (such as the ancestors of gallwasps and of many other small gall-inducing wasps). A general truth seems to be that tapping the resources of a plant more effectively, by inducing a gall on it, has been favoured by selection many times. This is one reason for suspecting that even if galling insects influence the same key system in plant development, the precise way they do it may be quite varied.

Related to this question is another: **Why are some plants galled and not others?** One very obvious pattern is that while some groups of galling insects (the best example is gall midges) have diversified to attack a very wide range of plants, others (such as gallwasps) attack a small number of very different plant groups. There are two possible explanations for this sort of difference. One is that gallmidges find it much easier to 'switch' (in evolutionary terms) from one group of plants to another than gallwasps do, perhaps because they target a more fundamental (and so widely shared) aspect of plant development. If gallwasps target a more specific plant developmental pathway, then the groups they attack (oaks, roses, and a narrow range of other woody plants and herbs) could be those sharing this specific metabolism. Clearly it will be much easier to judge this idea when we know more about how different insect groups induce their galls!

The second possible explanation is that gallmidges and gallwasps both switch between plant groups at the same rate, but that gallmidges have been around much longer. We may be able to answer this question before we know how gall induction happens. Methods now exist that allow us to estimate the ages of different groups of organisms using DNA sequence data. For example, it looks as if the ancestors of gallwasps now present in Europe diversified in Iran and Turkey some 8-10 million years ago, while DNA data and fossils suggest that gallwasps as a group are at least 50 million years old. What we need now is the same sort of historical dating study on gallmidges.

**Why do galls come in such amazing shapes and sizes?** It is clear that the main function of galls (from the gall inducer's point of view) is the provision of high quality food, free of the defensive toxins with which other plant tissues are defended (examples are caffeine and nicotine!). While this explains the inside of galls, it doesn't explain why many galls are so complex structurally, particularly on the outside. Galls clearly do protect their inhabitants from weather, and also from natural enemies. A validation of these arguments comes from the speed with which some galling aphids repair their galls if a hole is made (for example, by a caterpillar). The aphids crawl into the breach and explode, rapidly plugging the gap with a form of 'fibreglass' made from a latex that they store within their bodies, and their own corpses (you can see more on this following the web-link below). The aphids then tend the repair, stimulating plant tissues to grow and so reseal the breach.

Some structural attributes of galls have obvious protective functions. Some oak gallwasp galls secrete honeydew, which attracts ants that then kill or drive away any parasitic wasps trying to attack the gallwasp larva (see the Japanese nectar-secreting species *Andricus hakonensis* in Plate 3). Oak trees do not naturally secrete nectar (they are wind-pollinated), and within the gallwasps this very specialised form of bodyguard defence has evolved at least 6 times. Other attributes of galls look defensive to us – coatings of pointed spines or sticky resin, for example. However, the impact of most gall structures has yet to be tested experimentally. We need to see how gallwasp enemies – such as parasitoid wasps (such as the beautiful *Torymus auratus* in Plate 4) or birds - deal with different galls, or interpret the bright colours that we often see in oak galls and make them easier to see for human eyes. Only then will we be able to understand how natural selection and history have shaped gall diversity. However, this will be quite a challenge! Unlike fruitflies, most gallers and their associated enemies are very hard to maintain in culture, and so the experiments we can design easily enough on the back of an envelope in the pub are very hard to do in practice.

**How can I tell all the inhabitants in a gall apart?** Many scientists work on the communities of insects inhabiting galls. Because galls can easily be collected and their inhabitants reared, they make excellent 'study systems' for looking at a whole range of ecological processes - in our case, particularly the way in which invading gallwasps (such as the knopper gallwasp) have accumulated enemies after their arrival in Britain. Identifying these inhabitants may not seem like an obvious challenge – after all, identification keys based on adult characters have been around for many gall inhabitants for a long time. However, many of the inhabitants of galls that look identical – even to experts such as Dick Askew – have recently been shown to be members of different (but indistinguishable!) species. Genetic data show that many of the parasitic wasp species found in oak gallwasp galls are actually groups of indistinguishable species that separated up to 10 million years ago! It seems that when these parasites split into new species, aspects of their biology change while their overall shape and size (and so the characters we use to distinguish them in keys) often don't. Genetics has shown the situation in some other groups to be even more confusing – with the inquiline gallwasps (*Synergus* and relatives) being the worst! Here we not only have different species that look the same

– we also have specimens that look different (and are currently classified as different species) actually being the same species!

So do these alterations to the long-accepted order of things matter? Well, they do if we want to understand how the communities of animals associated with galls work. For example, one common species can evolve in a very different way to a set of separate, less abundant (but indistinguishable) species, and *vice versa*. The best way to resolve these issues is to use DNA sequence information, a technique known as ‘DNA barcoding’, which can tell us reliably whether a group of similar-looking specimens are one species or several. But does this mean the end of amateur natural history exploration of galls and their inhabitants? We hope not! Our hope is that even though existing keys and characters definitely don’t work in some cases, once DNA barcodes confirm who is who we can search for new morphological characters that we can use to build new keys. There is hope that such new characters need not be visible only with an electron microscope. A whole new class of characters has recently been developed, based on generating rainbow thin-film patterns from wings. Against a dark background, light shone on transparent insect wings produces a pattern similar to the colours seen in a soap bubble or film of oil. The patterns you see depend on the thickness of the wing between the upper and lower membranes, and these patterns can distinguish otherwise very very similar specimens. These ‘thin film’ colours are also easy to see, and so should be usable by academics and amateurs alike. We are currently trying to develop these and other new characters for oak gallwasp communities.

There are other advantages of exploring a genetic view of taxonomy. While different lifestages of the same species have very different morphologies, they all have the same genes, and so once we have a DNA barcode for a verified adult of any species, we can also identify eggs, larvae and pupae, even when these are very small, and even cast skins or corpses. This should make it easier to identify the makers of mystery galls, or the killers of other gall inhabitants as long as we can find a body part whose DNA we can sequence!

Cornell HV (1983) The secondary chemistry and complex morphology of galls formed by the Cynipinae (Hymenoptera): Why and how? *American Midland Naturalist* vol. 110: 225–234.

Kutsukake M et al (2009) Scab formation and wound healing of plant tissue by soldier aphid. *Proceedings of the Royal Society of London Series B* vol. 276 no. 1662 1555-1563

Kurosu U et al (2003) Self-sacrificing gall repair by aphid nymphs. *Proceedings of the Royal Society of London Series B* vol. 270 no. Suppl 1 S12-S14

Suicidal aphids web-link

<http://blogs.discovermagazine.com/notrocketscience/2010/06/17/suicidal-menopausal-aphids-save-their-colony-by-sticking-themselves-to-predators/>

### **Plate legends.**

Plate 1. The very first stage in the development of a Knopper gall of the gallwasp *Andricus quercuscalicis*. The insects are female knopper gallwasps, laying their eggs into a fertilised female flower of English oak, *Quercus robur*. The flower, which would usually now develop into an acorn, will develop instead into the knopper galls familiar to BPGS members. Photo Gyuri Csóka.

Plate 2. A Freshly emerged *Asphondylia sarothamni* gallmidge on its gall. Induced on broom (*Sarothamnus*). Photo Gyuri Csóka.

Plate 3. An ant sipping nectar secreted by an asexual generation gall of *Andricus hakonensis*, induced on *Quercus dentata* in Japan. Photo Gyuri Csóka.

Plate 4. A female of the parasitoid *Torymus auratus* ovipositing into a sexual generation 'oak apple' gall of the gallwasp *Biorhiza pallida*. Photo Gyuri Csóka.