

What are the big challenges in sleep research? Most research indicates that sleep is an essential part of daily life. When we lose sleep, our ability to interact adaptively with the environment becomes compromised. This ranges from lapses in attention while listening to a lecture to falling asleep while driving a car. Sleepiness is even implicated in large disasters, such as the Challenger space shuttle explosion, the Exxon Valdez oil spill, and the nuclear power plant accidents at Three Mile Island and Chernobyl. Decreased waking performance is not unique to humans; even dancing bees lose a step after losing sleep. Impairment arises when the homeostatic pressure to recoup lost sleep causes parts of the brain to fall asleep. In addition, the wear and tear of prolonged wakefulness might impair neuronal performance.

Surprisingly, our recent studies on pectoral sandpipers and great frigatebirds suggest that reduced performance is not an evolutionarily inescapable outcome of sleep loss. In collaboration with Bart Kempenaers (MPIO), John Lesku (a PhD student in my lab), and many others, we found that polygynous male pectoral sandpipers that sleep the least are the most successful at siring young, a difficult task that entails out-competing other males for a territory and successfully courting choosy females who decide whether to put all their eggs in a particular male's (genetic) basket. Although it is possible that this short-sleep strategy incurs long-term costs, higher paternity suggests that sleep loss did not have the immediate and cumulative adverse impact on waking performance that is typically observed in other animals. Similarly, in collaboration with a large team, including Alexei Vyssotski and Martin Wikelski (MPIO), my postdoc, Bryson Voirin, and I recently found that, even though female great frigatebirds can sleep in flight, and usually do so unihemispherically like the ducks, they actually sleep less than an hour per day during week-long foraging trips that are spent entirely on the wing.

Reconciling this dichotomy between the wealth of evidence demonstrating decrements in performance resulting from sleep loss, on the one hand, and the ability of some birds to forgo amounts of daily sleep that would render us incapable of safely driving a car,

on the other, is an exciting challenge for the sleep research field. Increased motivation, mediated by the release of the wake-promoting neurotransmitter dopamine and/or neuropeptide hypocretin, might sustain adaptive waking performance by keeping the homeostatic pressure for sleep at bay. However, if simply putting the brakes on the homeostat is all that is needed to keep adaptive wakefulness going, this would suggest that neuronal wear and tear caused by wakefulness has no impact on performance. Although this might be correct, it is also possible that unknown resources are summoned to repair wear and tear on the go and thereby prolong periods of adaptive wakefulness.

My own personal experience questions whether the ability to perform adaptively on little sleep is simply a function of motivation. While driving to a site in Panama to study frigatebirds, I was caught out on the road late at night by myself. I had planned to make the long drive through this beautiful country during the day, but I got delayed by an auto accident (not my fault) in Panama City and later by a speed trap manned by a friendly police officer who escorted me to an ATM when I failed to produce the appropriate toll. While I was not aware of the speed limit, being a sleep scientist I was acutely aware of the dangers of driving well past my bedtime. However, I did not feel safe sleeping alongside the road. So, I pressed on, desperately trying to stay awake, wishing I was a duck. When I woke up driving on the wrong side of the road, I decided that stopping to sleep was the safer option. Clearly, being motivated to stay awake by the dangers of falling asleep at the wheel was insufficient. Consequently, I suspect that motivation alone does not explain how frigatebirds stay awake throughout most of the monotonous nighttime hours spent gliding over the ocean. Ultimately, determining how some birds are able to perform adaptively on little sleep might inspire a new understanding of sleep's functions, as well as novel approaches to mitigate the consequences of insufficient sleep in humans.

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Quick guide

Cynipid gall wasps

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What are cynipid gall wasps?

Symbiosis describes any intimate, long-term interaction between two different biological species, which can range from mutualism to commensalism to parasitism. Gall-forming insects perform one of the most fascinating examples of parasitic symbiosis found in nature, where they induce undifferentiated host plant cells to form nutrient-rich, tumor-like growths called 'galls'. These insect-induced plant galls serve as microhabitats within which larvae feed, develop and pupate. Remarkably, the galling lifestyle has evolved independently in six insect orders: Diptera (flies), Hymenoptera (bees, wasps, and ants), Coleoptera (beetles), Lepidoptera (butterflies and moths), Thysanoptera (thrips) and Hemiptera (true bugs) and is described in greater than 20,000 insect species. An estimated 15–50% of vascular plant species worldwide are susceptible to galling insects, including agriculturally important plants such as grape (*Vitis*), wheat (*Triticum*), rice (*Oryza*), and blueberry (*Vaccinium*), where they can cause substantial reductions in crop yield.

Cynipid gall wasps comprise one of the most charismatic, species-rich, and phenotypically variable groups of galling insects with ~1,400 described species and many species still likely to be discovered (see below). The study of gall wasps has a long and rich history. Curiosity in gall formation can be found in early Chinese and Greek writings. Iron gall ink, which was the most common ink used from the Middle Ages to the 19th century, was used in line drawings by Da Vinci, Van Gogh, and Rembrandt, and in the writing of many historical documents such as the US Declaration of Independence. The study of gall wasps in the US included pioneering 19th century female naturalist Mary Treat (1830–1923), who exchanged letters with Charles Darwin, and a young Alfred Kinsey, who, before embarking on the study of human sexuality, traveled the southern



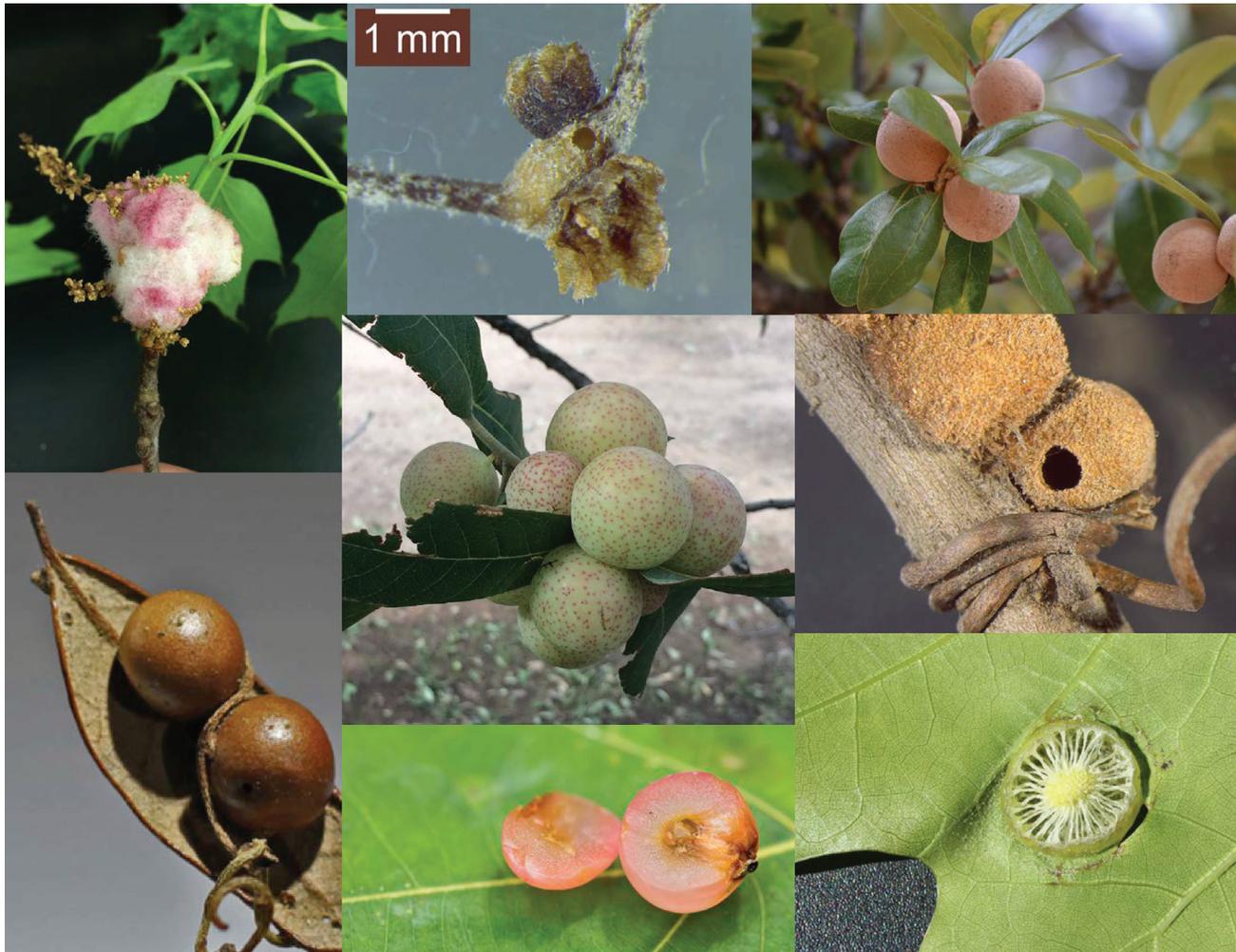


Figure 1. Galls induced by cynipid wasps on their oak tree host plants.

Highly variable, tumor-like galls induced by cynipid wasps on their host oaks, including a fuzz-covered gall induced by *Andricus seminator* (top left), a cryptic catkin gall induced by *Andricus quercuslanigera* (top middle), large spherical galls reaching 25–30 mm in diameter induced by *Disholcaspis cinerosa* (top right), a cluster stem gall that produces a sugary exudate induced by *Disholcaspis quercusvirens* (middle right), dissected leaf gall of *Dryocosmus quercuspalustris* showing the larval chamber and open air pockets (bottom right), dissected spherical gall of *Amphibolips nubilipennis* showing central chamber and solid internal structure (bottom middle), spherical leaf galls induced by *Belonocnema treatae* that are being attacked by a parasitic vine (bottom left), and a gall that is unknown to the authors found in the Chiricahua Mountains in southern Arizona (center panel). (Photo credits: Ellen Martinson, Mattheau Comerford, and Adam Fenster.)

US and Mexico between 1915 and 1925 amassing a collection of over 7.5 million cynipid wasps, their galls, and natural enemies, now preserved at the American Museum of Natural History in New York City.

Gall wasps are part of a larger evolutionary group within the Hymenoptera called the Cynipoidea, which is a lineage dominated by parasitoids that attack insect larvae. This affiliation suggests that gall induction on plants evolved from a common ancestor that parasitized other insects. Most cynipids (~70%) gall oak trees in the genus *Quercus*

(Fagaceae), with a smaller number of species galling other plants, including chestnuts, roses, and even some herbaceous plants. Each species of gall wasp typically attacks a single or closely related group of plant species and induces a morphologically distinct gall on a single plant tissue, such as roots, stems, buds, leaves, petioles, fruit or flowers (Figure 1). These galls can be single- or multi-chambered, housing one or multiple developing wasps. Interestingly, a cheater lineage of gall wasps in the tribe Synergini have lost the ability to initiate gall formation, but develop as inquiline —

an organism that exploits the living space of another — within the galls induced by other species. One major strength of gall wasps as a study system is that individual host plants are often simultaneously or sequentially galled by multiple cynipid species. This allows for behavioral, ecological, genetic, or developmental comparisons of multiple distinct lineages of galling wasps that manipulate the same host oak genome to create species-specific gall phenotypes. Moreover, each gall is itself host to a diverse community of natural enemies that make gall wasps an important study system for



Figure 2. The cynipid wasp and its enemies.

The cynipid gall wasp *Belonocnema treatae* (top left) and many gall-wasp-associated natural enemies, including the crypt-keeper wasp (*Euderus seti*; top right), a Pteromalid parasitoid in the genus *Cecidostiba* (middle right), a beetle inquiline in the genus *Tricorynus* (bottom right), and a Eupelmid parasitoid in the genus *Brasema* (bottom left). (Photo credits: Andrew Forbes/University of Iowa.)

addressing questions in community ecology (Figure 2).

How do gall wasps induce galls to form in plants? Gall formation is a form of symbiosis wherein the insect evades host external defenses and internal immunity, and co-opts molecular control of plant tissue development to create a novel organ for their own purposes. Insect-induced galls are considered an ‘extended phenotype’, in that the initiation, development, and maintenance of galls are controlled by the insect, but the gall itself is composed entirely of plant tissue. These structures are commonly unique to the host plant in form and sometimes even color — appearing nowhere else on the host during their life cycle. Interestingly, we still know very little about how galls are induced, the signaling involved or whether induction mechanisms are consistent across independent gall-forming insect lineages. The most common proposed signaling involves plant hormones such as auxin; however, studies have never been able

to explain the complexity of cynipid galls by phytohormones alone. What is known is that the plant interacts with various wasp secretions throughout the three stages of gall formation: initiation, growth and maturation. Gall initiation occurs after the wasp inserts (i.e., oviposits) one or more eggs, as well as a maternal secretion from the venom gland, into meristematic tissue of the host plant. In response, the plant cells adjacent to the egg undergo apoptosis, with the remaining tissue proliferating and differentiating into wound callus-like cells. The maternal secretion injected into the plant derives from the same gland that the ancestral parasitic wasps used to manipulate the metabolism of their insect hosts. Thus, it is thought that cynipid venom has evolved to play an important role in gall initiation, though the signaling between the wasp and host plant is currently not known. The growth stage is marked by rapid cell growth and differentiation. Nutritive cells form the inner larval chamber of the gall, then a secondary layer of tissue develops, which connects to the host’s vascular

network and differentiates into species-specific structures of the outer gall cortex. Histological studies of gall structure have noted that whereas the outer gall cortex is species-specific, the organization of the inner larval chamber is generally conserved in most species. A recent study has shown that the wasps significantly alter the expression of nearly a third of the oak genome between the inner gall tissue and the leaf tissue from which it was derived, which indicated that the gall wasp is not merely modifying oak leaf tissue but creating a novel organ on its host plant. If larvae are killed during this phase, gall growth is halted. Thus, it has been suggested that the active chewing and larval salivary gland secretion are also essential for gall growth. This also indicates that cynipid wasps do not use the same method for gall initiation and growth as the bacterium *Agrobacterium tumefaciens*, which creates crown galls by inserting T-DNA into the plant genome that can continue gall growth even if the bacterial infection is cleared. The mature phase of the gall is marked by cessation of growth, lignifying of the outer gall tissue, and pupation of the wasp larva, which occurs when the signaling between the wasp and plant ceases.

How do wasps benefit from galls and why are galls so variable? Through their galls, gall formers receive nutrition from their host plant, protection from environmental conditions, and defense against predators. Each of these factors has played a role in the origin of gall formation, as well as the evolution of the great diversity of gall morphologies. In fact, the morphological variation among cynipid galls is quite staggering — sticky or hairy surfaces, size and color variation, spiked or thick walls, false chambers, etc.! The leading hypothesis for the variation in gall morphology is protection against natural enemies. Gall wasps harbor large communities of natural enemies, which can inflict high mortality (sometimes as high as 99%). Studies on the impact of intraspecific variation in gall traits on gall wasp survival provide compelling evidence that some traits significantly reduce mortality from natural enemies. These traits include the recruitment of ants and other insects with a sugary

exudate, overall gall size and chamber number, and external surfaces covered in hairs (plant trichomes).

How do gall wasps reproduce and what is cyclical parthenogenesis?

Gall wasps have become models for studying the evolution of sexual reproduction. Cynipid gall wasps reproduce via cyclical parthenogenesis, which involves the alternation of sexually and asexually reproducing generations to complete a typically annual bivoltine life cycle. This unique life cycle differs from most cyclically parthenogenetic organisms, such as aphids or *Daphnia*, which primarily reproduce asexually with sexual reproduction induced by fluctuations in environmental conditions. In gall wasps, the alternation of sexual and asexual generations is obligate: the sexual generation mates and generates the asexual generation. Individual asexual generation females then either produce haploid males or diploid females parthenogenetically. The genetic mechanisms of sex determination in this system are complicated, largely unknown, and represent an exciting area of current and future work.

However, the cyclical parthenogenetic life style is also advantageous for scientific inquiry. First, this complex life cycle requires the adaptation to and use of different environmental conditions where the gall wasps of each generation are subject to different suites of natural enemies, as well as biotic and abiotic challenges. For example, the asexual generation of the gall wasp *Belonocnema treatae* develops within single-chambered, woody, spherical leaf galls formed on the underside of newly flushed oak leaves in the spring and summer, whereas the sexual generation develops within multi-chambered, fleshy root galls formed on young root-shoots in the fall and winter. This raises the possibility that multiple axes of selection act differentially between gall wasp generations, but on the same genome. How the responses to selection is mediated by a single genome is an open and exciting question. Second, there is evidence in some gall wasp species that one of the two generations has been secondarily lost. In fact, many gall wasp species

are only known from their asexual generation, which offers the opportunity to explore questions regarding the evolution and maintenance of sexual reproduction. Along these lines, gall wasps, like most insects, are commonly infected with *Wolbachia*, the endosymbiotic Gram-negative bacteria that is a common reproductive parasite associated with parthenogenetic reproduction. However, populations of gall wasps species found to be infected with *Wolbachia* continue to produce males; thus, the impact of *Wolbachia* on gall wasp reproduction remains an open question. One study of the gall wasp *B. treatae* found variation in *Wolbachia* strain type and infection frequencies between populations, and raised the potential role of *Wolbachia* in generating cytoplasmic incompatibility between gall wasp populations.

Why are gall wasp systems a source of undiscovered biodiversity?

Gall wasps exhibit a global distribution, where they are found on every continent except Antarctica. Interestingly, global estimates of gall wasp species are still imprecise for many reasons. On a species level, many regions that are predicted to be the most biodiverse remain underexplored. Gall wasp species diversity is strongly associated with the diversity of their host plants in well-characterized areas. Thus, it stands to reason that regions with a high potential host diversity may exhibit high species richness for gall wasps as well. Specifically, two regions are notable: firstly, Mexico and Central America, which is one of the most species-rich areas for oaks (genus *Quercus*) and, secondly, the oak forests in Southeast Asia, Japan, and China, which have not been intensively sampled (yet).

Within gall wasp species, the alternating generations of many cyclically parthenogenetic cynipid species have yet to be discovered. Thus, there are many opportunities to describe a new form of biodiversity within a previously described species, which taxonomists occasionally refer to as a ‘paralectotype’. In one recent example, we described the previously unknown sexual generation of the gall wasp *Andricus quercuslanigera*, whose asexual generation induces fuzzy single-chambered galls on the

undersides of newly flushed leaves of its host plant, *Quercus virginiana*. By combining natural history, detailed sampling of potential host tissues, behavioral observations, and DNA sequencing, we found that the sexual generation develops within the highly ephemeral oak catkins (i.e., male reproductive structures) that grow and fall off the tree within 2–3 weeks time. Interestingly, while sequencing the DNA of the newly discovered sexual generation, we genetically uncovered a completely different lineage of gall wasps, for which we are now searching for other alternative generations!

Finally, on an ecosystem level, cynipid gall wasps are considered ecosystem engineers in that they significantly modify the environment in a way that has a large impact on the species richness and heterogeneity in an area. Primarily, this is due to the predictable, well-defined, and closed system that the gall generates. Galls are typically attacked by a large community of natural enemies throughout growth and development, and even long after the gall former has emerged. These communities include inquilines, parasitoids, and predators. Inquilines consume plant material in the gall and can kill the gall former directly or indirectly. Inquilines include the cheating cynipid lineage (Synergini), as well as other herbivorous insects such as moths and beetles. Parasitoids directly attack and consume the gall wasp, and gall communities can range from 2 to greater than 30 different species of parasitoids associated with a single gall wasp species. One unanswered question is the degree of specialization in this guild of natural enemies. Historically, they were thought to be more generalists; however, careful natural history, observation and molecular genetic work argues that some parasitoid lineages are highly specialized. In addition, there can be high mortality due to predation, which includes insect consumers eating both plant and gall wasp tissues, as well as vertebrate predators, including many birds, such as woodpeckers and chickadees, and rodents, including squirrels and rats. Vertebrate predation of gall formers is still an understudied aspect of these species-rich natural enemy communities. Lastly, many other taxa can attack galls and gall formers,

including plant endophytic fungi and even parasitic plants, such as the love vine *Cassytha filiformis*, which has recently been found to attack galls and influence gall wasp fitness. This complex system of natural enemies has made gall former communities a model system for questions in community ecology.

How can the study of gall wasps contribute to other areas of science?

Cynipid gall wasps manipulate the stem cells of their host plants and exhibit bizarre and complex life cycles. They are distributed globally, attack most lineages of vascular plants, and exhibit an amazing array of phenotypic diversity. The structurally complex tumors cynipids induce harbor a species-rich community of natural enemies from which new taxa are discovered each year. Lastly, the genomes of many of these interconnected organisms are currently being sequenced with the hope of unlocking some of the secrets this charismatic group conceals. The question is not what areas of science can the study of gall wasps contribute to, but what areas of science can the study of gall wasps not contribute to?

Where can I find out more?

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Primer

Plasmodesmata and the symplast

Christine Faulkner

Multicellular organisms rely on cell-to-cell communication and resource exchange to coordinate the various diverse processes involved in growth, development, and environmental responses across tissues and organs. Most complex multicellular organisms have highly organised and specialised anatomies, which develop by processes underpinned by regulated mechanisms for intercellular coordination. Indeed, in 1897 Wilhelm Pfeffer noted that for a plant to coordinate its physiological responses across the whole, there must be continuity throughout the entire organism, and that connections between cells must transport material and messages between tissues. Intercellular communication is an integral factor in any tissue-wide or organ-wide process in a multicellular organism.

Like other complex multicellular organisms, plants have cells with distinct identities and functions, and these are organised into tissues and organs. Remarkably, different plant organs are exposed to vastly different environments depending on whether they are aerial or subterranean, underlining the critical need for communication to mediate coordination within a plant. Further, unlike animals, plants do not have mobile cells, and therefore the mechanisms by which plants transport resources and signals between tissues must rely solely on structural connections between cells. Plant cells are surrounded by a cell wall that prevents immediate contact between the membranes of neighbouring cells. To breach the cell wall barrier, plants have specialised plasma membrane-lined tubes called plasmodesmata that connect adjacent cells. These tubes create an interconnected, multicellular cytoplasm that traverses almost all tissues of the plant and is referred to as the symplast. Plasmodesmata connect the symplast directly to the phloem, which serves as a long-distance conduit within the vasculature and allows rapid

translocation of material between tissues. Thus, plasmodesmata allow both short distance and long distance molecular transport throughout a plant. Applying the assumption that any soluble molecule that is small enough to fit through a plasmodesma can freely move through the symplast, we can infer that many molecules travel long distances via this pathway to carry both resources and signals between cells and tissues.

Plasmodesmal structure

Structurally, plasmodesmata are plasma-membrane-lined tubes, or tunnels, that cross the cell wall and generate cytoplasmic and plasma-membrane continuity between neighbouring cells (Figure 1). When the cytoplasm of one cell is loaded with a fluorescent dye, the dye moves freely into neighbouring cells, demonstrating the capacity of the cytoplasmic connections to facilitate molecular exchange between cells. By contrast, the plasma membrane that passes through a plasmodesma does not seem to function as a transport pathway for lipid-soluble molecules, despite its continuity between neighbours.

Electron micrographs show that there is also a rod of endoplasmic reticulum (ER) membrane that runs through a plasmodesma, connecting the ER of adjacent cells. This membrane rod is known as the desmotubule (Figure 1B). Dyes and proteins that are soluble in ER membrane have been seen to move between cells, but the same is not true for molecules contained within the ER lumen. This correlates with electron micrographs of cross sections of plasmodesmata in which the desmotubule appears as a tightly appressed membrane tube with no space for functional lumen.

Cross sections of plasmodesmata show that there are connections, or spokes, that link the desmotubule to the plasma membrane. The identity of these spokes has been a topic of debate since they were first observed, but a recent hypothesis is that they are membrane contact sites. According to this idea, the spokes would be calcium-responsive proteins that are anchored in both the ER and the plasma membrane. How they function to define plasmodesmal structure and function is not yet clear, although

