

A close-up photograph of a bumblebee on a blue flower. The bee is the central focus, with its fuzzy black and yellow body and transparent wings clearly visible. It is positioned on a vibrant blue flower with several petals. The background is a soft, out-of-focus green, suggesting a natural outdoor setting. The lighting is bright, highlighting the textures of the bee's fur and the flower's petals.

ONE TREE
TO BIND THEM

by J. William Bell

A comprehensive history of bumble bee evolution—completed with the help of NCSA—cuts against the conventional wisdom on how the insects' color patterns and social behavior developed.

You've probably been admonished not to miss the forest for the trees. But for those building phylogenies, trees recover the ancient evolutionary story of how organisms are related to one another. According to entomologist Sydney Cameron, adding branches (that is, species) to these trees increases the value of these systematic arrangements of species, based on their genetic makeup. "The inferences are much more powerful with more species included. Having all the species of an entire genus—or virtually all—is phenomenal," says Cameron, a professor at the University of Illinois at Urbana-Champaign.

In an upcoming issue of *the Biological Journal of the Linnean Society*, Cameron and her collaborators reveal the first comprehensive phylogeny of the genus *Bombus*, also known as the bumble bee. Collaborators include graduate student Heather Hines and Natural History Museum of London entomologist Paul Williams. Their tree includes nearly 90 percent of all 250 species represented in the genus worldwide.

"This is one of the few, if any, insect DNA phylogenies that includes almost every species in a large genus. A decade ago, it would have been a pipe dream," she says. "The interplay between [gene] sequencing technology, its cost, the algorithms involved, and the computational power—these have all come together recently to allow such a large scale study."

The computational power, and the expertise to harness it, came from NCSA. The team used the center's IBM p690 system to estimate the phylogeny of 220 species covering about 35 million years of evolution by comparing DNA sequences from five genes for each species. NCSA staff, meanwhile, assisted with debugging and writing code to manage the simulations.

The bumble bee tree is only now hitting the presses, but it has already stimulated new research on the timing of the bees' genetic divergence and ancient movements across continents. It has also led to new research into the evolution of their color patterns, which converge across distantly related species as a means of protective mimicry.

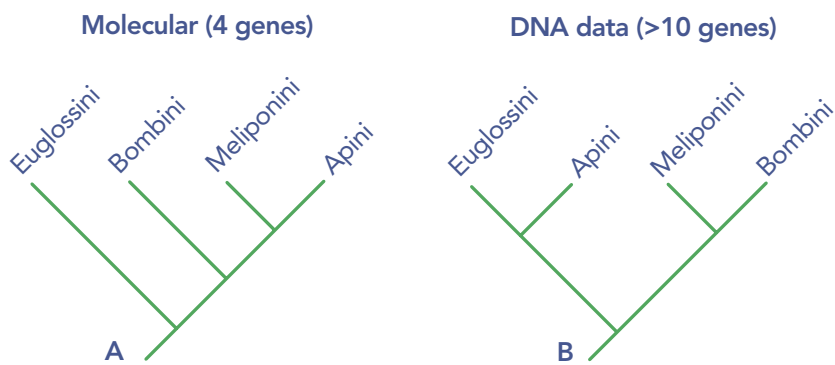
Bees as social climbers

Less than three percent of the approximately 20,000 known species of bees are classified as highly social; you see them on the quintessential Discovery Channel documentaries, with their strict division of labor centered around an egg-laying queen. The bumble bees of interest to the Cameron team are less hierarchical. They're classified as intermediately social, and the hives are less focused on the queen than are honey bees. But they're not the kind of bee to just lay their eggs and leave, never to see their offspring once they've hatched, as solitary bees do. This position in the middle of the social ladder between highly social and solitary bees makes them a particularly useful target of study.

Traditionally, highly social behavior was thought to be the pinnacle of evolutionary development. This implied a solitary common ancestor that divided into related but distinct species. Over millions of generations, some species maintained their solitary ways, while new species slowly accrued increasingly complex social behavior. Thus, the highly social bees were seen as the apex, having passed as earlier species through previous grades of sociality.

The Cameron team's studies of bee relationships, including the comprehensive bumble bee tree, contradict that thinking. The bumble bee branch does not sprout from the trunk between the solitary

A member of the *Bombus* genus. Cameron and her collaborators created the first comprehensive phylogeny of the genus, also known as the bumble bee.



Relationships between four tribes of corbiculate bees (honey bees, stingless bees, orchid bees, and bumble bees). This illustration shows the differences between phylogenies based on morphological data and those based on DNA data. The bumble bee branch (Bombini) does not attach between the solitary Euglossini, near the base, and highly social honey bees (Apini) and stingless bees (Meliponi) at the top, as previous theories looking at morphology would imply. Instead, according to multiple independent DNA studies, the highly social stingless bees (Meliponini) are most closely related to the intermediately social bumble bees (Bombini) and relatively distantly related to the highly social honey bees (Apini).

bees near the base and highly social honey bees and stingless bees together at the crown, as previous theories looking at morphology would imply. Instead, according to the DNA studies, the highly social stingless bees are most closely related to the bumble bees and least closely related to their highly social cousins, the honey bees.

“This gives us a completely different perception of the evolution of social behavior,” says Cameron. “Until recently, studies of the bees’ physical traits and their behaviors showed us a progressive series of steps toward a pinnacle of social behavior. Now we see it’s not a single pinnacle at all. There’s no single origin of highly social behavior. Maybe it can happen many times. It certainly has happened twice here.” Moreover, in a paper currently in press in the *Proceedings of the National Academy of Sciences of the USA*, Hines, Cameron, and colleagues have concluded a similar pattern of social evolution among wasps, showing that sociality also evolved twice independently in stinging wasps.

From these insights, the team and others like them are beginning to explore what makes something social. Environment, given the bees’ various locations around the world and how that relates to their social behavior, is thought to be significant. But entirely different pressures also likely play a role. Whole-genus and higher-level phylogenetic trees like those developed by the Cameron team are key to accurately teasing out gains and losses of traits, including different traits of social behavior and what might have caused them.

Upcoming work at NCSA will combine into a single large analysis comprehensive data from phylogenies of several genera—bumble, honey, orchid, and stingless bees. “We can see interesting things in smaller tests. But as these phylogenies become larger, the evidence is that much more accurate,” says Cameron.

A clever mimic

Bumble bees are also of interest to entomologists because of the striking degree to which they exhibit Müllerian mimicry.

With some forms of mimicry, harmless species adapt to take on the physical traits of a species that is noxious. A tasty plant may come to look like a poisonous plant over time, for example. In Müllerian mimicry, harmful species—different species of bumble bees

with their stingers—look like one another. This increases the pool of bees marked as dangerous to their predators and speeds the rate at which those predators figure out the threat.

It might be reasonable to assume that insects that share a color pattern are closely related genetically; that they all came from a common ancestor from which other traits diverged, but that they kept their similar markings. The comprehensive phylogeny of bumble bees shows conclusively that those with near identical striping patterns are usually distant relatives.

A particular black and orange pattern, for instance, “crops up all over the tree [in distant relatives]. But when you look at a distribution map of where all the bees with that black and orange color pattern are found, you see that they cluster in a geographic region,” according to Cameron. Entomologists are looking at other selection pressures, besides protection from predation, that might influence their coloring. For example, differences in climate and other environmental factors might be involved.

Their Bayesian best

In Cameron’s studies, millions of possible trees with different branching patterns are constructed during the computer analyses of species relationships, representing different possible ways all the species in a study could be related. Out of these millions of possible trees, the one thought to represent the most accurate tree based on a variety of criteria is chosen.

Until relatively recently, the data used in phylogenetic studies were mostly morphological characters, and the datasets were small. In Cameron’s studies, the data include nucleotides from the DNA sequences her lab generates. The datasets are much larger, comprising thousands of nucleotides, compared to the few hundred characters generated by studies of morphology.

Today, computational power—like that available at NCSA—and advanced algorithms allow researchers to look at meaningfully sized collections of species, such as the whole bumble bee genus. Many researchers, like Cameron’s team, now use model-based approaches, which incorporate specific theories concerning how nucleotides of DNA change over time.

For example, based on statistical analyses of DNA sequences, researchers know that nucleotide changes in some regions and at some sites of a sequence change much more rapidly than others, and that different genes may change at different rates. They can account for these variations by using specific models with the appropriate model-based methods of phylogenetic inference.

Powerful computers and algorithms also allow the Cameron team to use Bayesian analysis, in which observations of the emerging most-likely tree are used to infer the probability that the next proposed tree in the analysis will be the best tree. With these inferences, the algorithm can concentrate on the most likely candidates and reduce the number trees that are considered. These Bayesian calculations are taxing computationally.

With Bayesian algorithms and hundreds of hours of NCSA computing time required for a large analysis, there were challenges for Cameron's team to overcome. NCSA's Sudhakar Pamidighantam stepped in to help.

Pamidighantam worked with the team over the course of months to get the Bayesian algorithms to work properly and effectively on NCSA's systems. He also wrote scripts to make the code run in parallel across many processors at a time, allowing multiple analyses. "We would not have this comprehensive tree today without him," says Cameron.

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Access Online: <http://access.ncsa.uiuc.edu/Stories/bombus>

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Worldwide phylogeny, as developed by the Cameron team. The phylogeny is estimated from mixed model Bayesian analyses of combined sequences from five gene fragments (16S, opsin, ArgK, Ef-1 alpha, and PEPCK). Subgeneric clades are individually color-coded and labeled with the subgeneric name. Values above branches are Bayesian posterior probabilities; values below branches are parsimony bootstrap values. Alternative resolution from parsimony analysis is shown as dotted lines. Outgroup branches (in grey) are represented by dashed lines and have been shortened for visual purposes. Graphic assistance with tree design came from Ben Grosser, Director, Imaging Technology Group, Beckman Institute.

