More Fun Than Fun: Beetles Lead Complex Social Lives in Dead Trees

24th November 2021

The ambrosia beetle Xyleborus affinis*. Reprinted from Dijkstra, M. (2020) Science & research news,* Frontiers*, November 4. Open access. Image: Peter H. Biedermann*

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This article is part of the 'More Fun Than Fun' column by Prof Raghavendra Gadagkar. He will explore interesting research papers or books and, while placing them in context, make them accessible to a wide readership.

- *Among species found under tree bark, perhaps the most unique are the ambrosia beetles.*
- *Many insects live off dead trees, but in most cases, only the larvae live in the wood; the adults fly about to feed and mate.*
- *The uniqueness of the bark beetles is that insects in all life stages live entirely in the wood.*
- *The ambrosia beetles, among bark beetles, are further unique: they don't feed on the wood itself but use it to cultivate fungi and eat* their *agricultural produce.*

W.D. Hamilton, one of the 20th century's most famous biologists, once wrote: "A dying tree opens a wide variety of habitats for colonisation by insects", a phenomenon he called "funeral feasts", or 'phytonecrophily' – literally, *a love of plant corpses*.

Indeed, a rich living world thrives under the bark of a dead tree, replete with many dark mysteries. As Hamilton points out, the richness of the insects in this unique habitat is not so much about the number of species, but the uniqueness of the species found there and nowhere else.

One uniqueness that particularly fascinated Hamilton was an unusual mode of sexual reproduction called haplodiploidy. In this system, both sexual reproduction and parthenogenesis coexist.

Fertilised, diploid eggs carrying two sets of chromosomes – one from the mother and one from the father – develop into females. In contrast, unfertilised haploid eggs containing only one set of chromosomes, from the mother, develop into males. Females mate and store sperm received from their mates in a sac called the spermatheca, and use it whenever they need to produce daughters. But they can also produce males by curtailing the flow of sperm from the spermatheca to the oviduct and thus prevent the egg being laid from being fertilised.

A much-discussed consequence of haplodiploidy is its effect on genetic relatedness. Because the males are haploid, their sperm are clones of each other, i.e. they have identical genes (relatedness = 1). However, the diploid females employ the normal reduction division to randomly distribute one set of chromosomes to each egg, making the eggs share only half of their genes (relatedness = 0.5).

Consequently, full sisters, who inherit one set of their genes from the egg and another set from the sperm, share 75% of their genes (relatedness = average of 0.5 and 1 = 0.75). It is important to note that the relatedness between full sisters is greater than between mother and daughter (0.5).

By contrast, full-siblings in us and other diploid sexually reproducing species, both sisters and brothers share 50% of their genes (relatedness = 0.5) because both males and females are diploid, and both eggs and sperm are produced by reduction division. Thus, the relatedness between siblings is the same as that between parents and offspring.

Hamilton famously speculated that this increased relatedness among sisters in haplodiploid species should facilitate the evolution of altruistic, sterile worker females who would find it genetically more advantageous to remain sterile and help *raise sisters* rather than *produce daughters*.

It also explains, Hamilton argued, why there are no male workers in haplodiploid species: males are only related to their sisters by 0.25. Even though subsequent research (including that from my research group) has cast doubt on the role of such genetic asymmetries created by haplodiploidy, there is an intriguing, as yet unexplained association between haplodiploidy and social evolution.

Another consequence of haplodiploidy that sparked Hamilton's interest was that it gives mothers control over the sex of their offspring: they could *choose* to produce sons or daughters as required, and tune the sex ratio among their offspring. Hamilton realised that this ability would come in handy for inbreeding populations.

R.A. Fisher, the father of statistics and one of the three architects of the modern synthesis (of evolution), had shown that a 1:1 sex ratio is expected in outbred populations. Fisher argued that if the sex ratio deviated from 1:1, the minority sex would be in greater demand in the competitive mating market. Hence parents will benefit from producing more of the minority sex until the sex ratio becomes equal and there is no further advantage.

Indeed, Fisher realised long before we invented the phrase 'evolutionarily stable strategy' (ESS) that a 1:1 sex ratio is an ESS: no population with an *unequal* sex ratio can invade a population with an *equal* sex ratio, and a population with an *equal* sex ratio can invade any population with an *unequal* sex ratio.

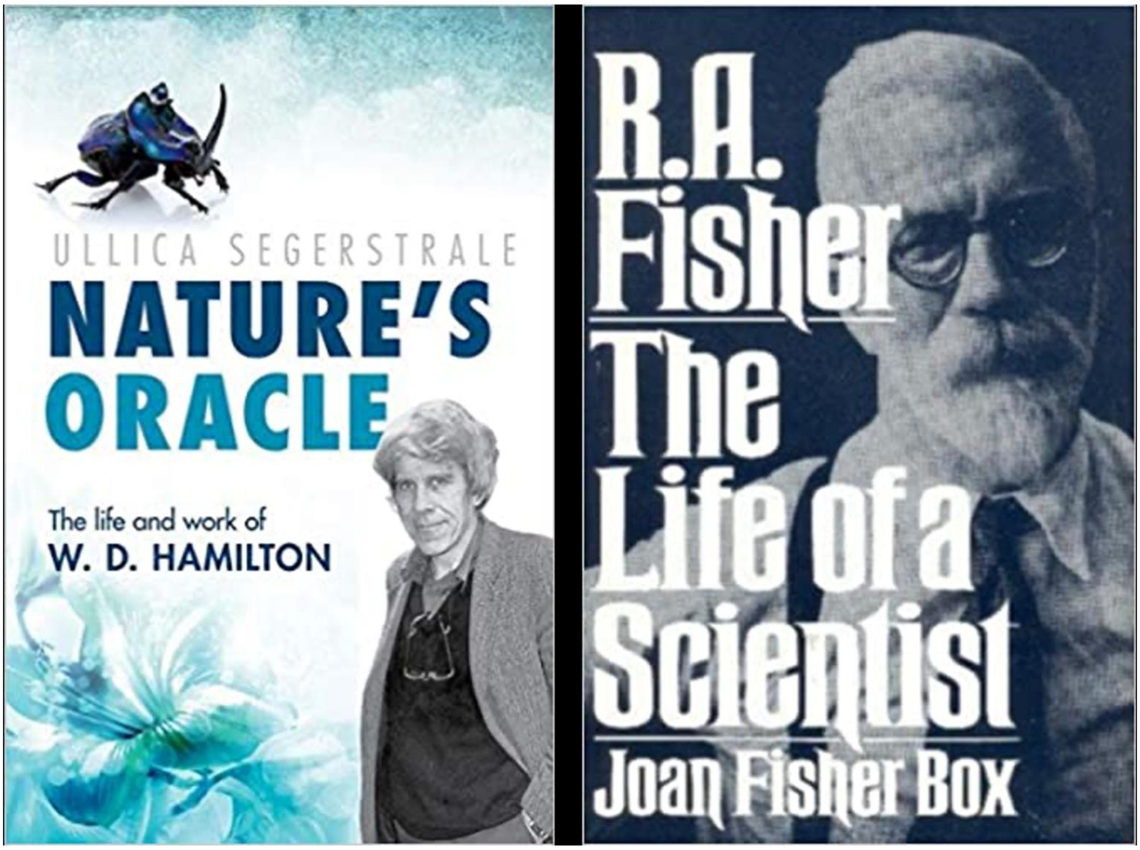
With his theory of 'local mate competition', Hamilton extended Fisher's theory in an important new direction. He realised that Fisher's ESS argument would hold only if there was population-wide competition in the mating market. But this wouldn't be true in an inbreeding population, where there would only be local mate competition.

In a paper with the revealing title 'Extraordinary sex ratios', Hamilton argued that because males would compete for mates only locally in inbreeding populations, the sex ratio would be expected to be female-biased. In the extreme case of complete brother-sister mating, a mother should produce only as many sons as necessary for inseminating all her daughters.

Hamilton then realised that haplodiploidy would permit mothers to adjust the sex ratios of their offspring as required under inbreeding. In an essay entitled 'Evolution and diversity under bark', he speculated that haplodiploidy itself would evolve more easily in inbreeding species. Because inbreeding is more likely in insects that spend their entire lives under the bark of trees, he also speculated that haplodiploidy is more likely to evolve *under the bark*.

Only now are we beginning to find evidence in support of Hamilton's visionary ideas. Researchers are studying some beetles belonging to the family *Scolytidae* to understand the origin of haplodiploidy – which, in fact, has *independently* arisen at least 15 times during the evolution of arthropods (insects, spiders, crustaceans and their relatives).

Incidentally, there are moving biographies of Hamilton, by Ullica Segerstrale, professor of sociology at the Illinois Institute of Technology, Chicago, and of Fisher by Fisher's daughter, Joan Fisher Box, that I heartily recommend.



Now, let us explore another kind of uniqueness under the bark. Among species found under the bark, perhaps the most unique are the ambrosia beetles. Many insects live off dead trees, but in most cases, only the larvae live in the wood; the adults fly about to feed and mate. The uniqueness of the bark beetles (family *Scolytidae*, in the insect order of *Coleoptera*) is that insects in all life stages live entirely in the wood.

The ambrosia beetles, among the bark beetles, are further unique in that they don't feed on the wood itself but use it to cultivate fungi and eat *their* agricultural produce. Even when they disperse from one tree to another, they carry an inoculum of the fungus to start a new fungal garden.

Attempts to reconstruct the phylogenetic tree of life of beetles suggests that this habit of cultivating fungi and living entirely in dead trees has been independently invented at least 11 times in evolutionary history. Let us pause to reflect that agriculture, which humans invented some 15,000 years ago and which was a major turning point in our civilisation, was developed by some ants, termites and beetles at least *50 million years ago*.

The fungi that ambrosia beetles cultivate are also unique and not found elsewhere. Indeed, the beetles get their name from the fungi, which are called ambrosia fungi. This is probably because the fungi are cottony white, sticky to touch and have a fruity odour, reminiscent of ambrosia, the 'food of the gods' in Greek and Roman mythology – a substance supposedly pleasing to touch and smell, and imparting immortality to its consumer.

There has been a long-standing interest, and much information has accumulated about ambrosia beetles because of their economic importance. Tiny though they are, they can still kill whole trees. But interest in the evolution of their remarkable parental and social behaviours and their agricultural practice is relatively recent, and our knowledge is quite sparse.

Those of us interested in social evolution must also take some of the blame for neglecting the beetles and many other 'lesser' social insects. We have erected an ivory tower of species showing the most complex possible social behaviour, such as in ants, bees, wasps and termites, called them eusocial, or 'truly social', and showered them with a disproportionate fraction of our attention.

A species has to show all three characteristics – reproductive division of labour, cooperative brood care and overlap of generations – to qualify as eusocial and thus become a favoured object of study. When James Costa reviewed what we then knew about non-eusocial social insects in 2006, he called his book *The Other Insect Societies*; when I reviewed his work, I called it *A Subaltern View of Eusociality*.

Most of us interested in the evolution of social behaviour woke up to the marvels of ambrosia beetles only when Deborah Kent and J.A. Simpson of the Forestry Commission in New South Wales, Australia, discovered eusociality in the ambrosia beetle (*Austroplatypus incompertus*) that lives on eucalyptus trees, as late as 1992. Since then, the reflected glory of that lone eusocial beetle has begun to brighten the scientific prospects of less than eusocial bark beetles.

Much of our information about the sociobiology of non-eusocial ambrosia beetles comes from the studies of Michael Taborsky and his students. Taborsky is a professor of behavioural ecology at the University of Bern, Switzerland. He has studied a wide array of animals, including various insects, spiders, fish, birds and mammals, to understand how and why diverse patterns of social and sexual behaviour persist in nature.

Along with Michael Cant of the University of Exeter in the UK and Jim Komdeur of the University of Groningen in the Netherlands, Taborsky recently wrote *The Evolution of Social Behaviour*. As I wrote in a blurb: "Breaking taxonomic boundaries and providing a smooth passage between theory, experiment and observation, Taborsky, Cant and Komdeur adroitly guide us through the fascinating world of social behaviour".

Michael Taborsky (left) and the author during the author's visit to Bern in February 2020. Photo: Geetha Gadagkar

Although I was pretty familiar with Taborsky's work, I was ignorant of his work on ambrosia beetles until I visited him in February 2020, just before the pandemic locked us all down. One of his students, Jon Andreja Nuotclà, or Deja for short, gave me a tour of his lab and showed me his laboratory colonies of the beetles.

I am always interested in why scientists choose to investigate a particular problem or study a specific species. So I asked Taborsky how he came to study ambrosia beetles. His answer, which I will quote in part, is instructive for students and mentors alike.

> "It all began with a failed project on the energetic costs of overwintering in rock ptarmigan (*Lagopus muta*). My then PhD student Katharina Peer, a keen birder originating from the Tyrolean high alps, hoped to study native birds in the mountainous landscape with which she was so familiar. I was interested in the energetics of behaviour and always wondered how rock ptarmigan benefit from collective overwintering.
>
> This turned out to be quite a job, in fact not really feasible for a single student in the time available for a PhD thesis. We had greatly underestimated the difficulty of catching these birds in the mountains – when I once helped her in the field, I realised that this was even more challenging than hunting down kiwis.
>
> After a year of hard trials, we decided to switch the subject. At that time, I was keen to find someone to study ambrosia beetles, a group that had grasped my interest for quite some time. Kathi was flexible enough to take over this topic, even if it diverged her from her fancied birds.
>
> She was a marvellous student and quickly developed laboratory settings allowing us to study the beetles' behaviour under seminatural conditions under the microscope while at the same time collecting field data in the forest surrounding the Hasli, to which we had recently moved from Vienna. All our subsequent work on these fascinating critters would not have been possible without Kathi's pioneering hard labour and intelligent achievements."

Michael Taborsky's students who pioneered the ambrosia beetle work – (left to right) Katharina Peer, Jon Andreja Nuotclà (Deja) and Peter H.W. Biedermann. Photos: Michael Taborsky

Taborsky and his students have focused their attention on a small number of non-eusocial ambrosia beetles. Katharina Peer first chose to study the ambrosia beetle *Xylosandrus germanus* in the Hasli Ethological Station in the Bremgarten forest in Bern. It is one of nine similar species introduced into Europe from East Asia about 50 years ago.

In this species, mated females hibernate during the winter and wake up in May-June. They then disperse, carrying some fungal spores, colonise freshly dead trees, bore into them with their mandibles to excavate a brood chamber (also called a gallery) and deposit the fungus.

Once the fungus grows using the decaying wood as nutrition, they lay eggs. These eggs are a mixture of male-destined unfertilised eggs and female-destined fertilised eggs. But in what proportion should they lay male- and female-destined eggs (remember that they have the power to choose)?

As we saw above, Hamilton's 'local mate competition' theory suggests that they should produce a very high proportion of daughters if their sons and daughters are only going to mate with each other.

But if their sons disperse and compete with other males to mate with other females, they should produce an increasing proportion of males as their sons increasingly outbreed.

Thus, there are two questions. First, what do the sons do – whom do they mate with, their sisters or others or a bit of both? Second, what do the mothers do – what ratio of sons and daughters do they produce?

Note that there is an added complication: the mothers have to decide on the sex ratio of their offspring even *before* their sons have had the opportunity to do whatever they do. Can these 2-mm beetle mothers predict what their sons will do in the future and control the flow of sperm from the spermatheca to the oviduct accordingly, to adjust the sex ratio of their progeny adaptively?

I must confess that this seems like a far-fetched expectation.

Peer and Taborsky set out to answer these twin questions. They studied beetles in the field by observing the behaviour of individually marked males and females. They found that males emerged from their pupal cases before the females, and at least some of them left the galleries of their birth and attempted to mate with newly emerged females from other galleries. Males were more likely to hang around galleries with many females, which they appeared to sense even without entering the galleries.

Their field observations suggested that mother beetles indeed produce a higher proportion of sons when they had higher mating opportunities outside the family. Apparently, the mothers could judge this by the density of galleries in the neighbourhood.

Snapshots of ambrosia beetle galleries in the field. Photo: Peter Biedermann

To confirm their suspicion, that mothers adjust the sex ratio of their offspring based on the outbreeding opportunities that their sons are *likely* to have, Peer and Taborsky conducted laboratory experiments. They placed one, two or three female beetles in test tubes with an artificial medium and simulated the conditions required to initiate new galleries.

The logic of the experiment was as follows. If there was only one mother beetle per test tube, her offspring would have no choice but to mate with each other (complete inbreeding). However, if there were two mothers per test tube, each mother's sons would have the opportunity to also mate with the female offspring of the other mother (at least partial outbreeding). If there are three mothers per test tube, the opportunities for outbreeding would be even greater.

As predicted from theory and as suspected from field observations, the proportion of sons produced by the beetles increased with the presence of other beetles in the test tube.

The expectation that mother beetles would be able to predict the future outbreeding opportunities for their as yet unborn sons and appropriately adjust the sex ratio of their offspring was not so far-fetched after all.

If natural selection had merely fixed a certain sex ratio based on the kind of outbreeding opportunities that always existed for a given species, I might have been somewhat less impressed. But the ability of the beetles to facultatively adjust the sex ratios of their offspring in real-time based on perceiving the current environment truly leaves me dumbfounded.

Next, Taborsky and his students turned their attention to the social life of ambrosia beetles. They monitored the galleries of the related species fruit-tree pinhole borer (*Xyleborinus saxesenii*) in Bern's forests.

They found that adult females may delay dispersing despite opportunities being available, and some may never disperse. So what do they do at home? There is growing evidence that non-dispersing females make themselves useful and thus help the colony of their mother grow, even though they may not reproduce.

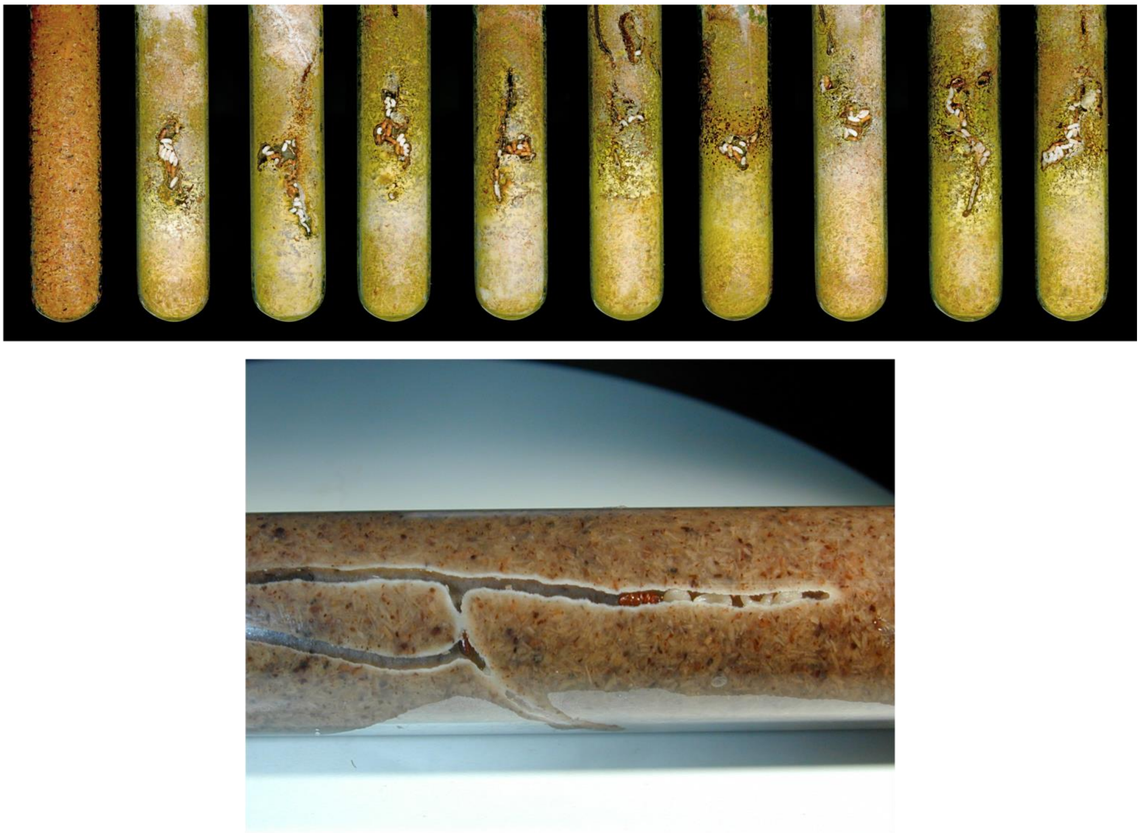
Ambrosia beetles appear to be ideally suited for such altruism to evolve. Their colonies consist of closely related individuals both because of haplodiploidy and because they are highly inbred. Dispersal is costly and risky, and it is relatively easy to make oneself useful at home – they can grow the fungus gardens, extend the galleries and clean and feed the larvae. These conditions can help them to get indirect fitness to more than compensate for the loss in personal reproduction.

In collaboration with two other students, Jon Andreja Nuotclà and Peter H. W. Biedermann, Taborsky discovered an even more important way non-dispersing females can make themselves useful.

Disease-causing pathogens are the scourge of all densely packed societies, and the galleries of ambrosia beetles are no exception. By experimentally infecting laboratory colonies of *X. saxesenii* with spores of the pathogenic fungus *Aspergillus*, they showed that a) the adult beetles increase their hygienic behaviours, such as grooming or cannibalising infected individuals; b) such increased hygienic behaviour helped control the infection; and c) that female beetles delayed their dispersal in response to infection.

Biedermann and Taborsky made a more detailed study of behaviour within the galleries of *X. saxesenii*, *focusing* on the division of labour. They recorded six behaviours that they called digging (extending the galleries), cropping (caring for the fungus garden), allogrooming (cleaning each other), balling (compacting waste), shuffling (getting rid of waste), and blocking (protecting from predators and preventing larvae from getting lost).

They found a new form of division of labour rarely found in ants, bees and wasps: division of labour between the adults and the larvae.



Snapshots of ambrosia beetle galleries in laboratory test tubes growing on artificial media. Photo: Peter Biedermann

The larvae of ambrosia beetles do their bit to maintain the galleries. The larvae specialise mainly in digging and balling; young females specialise in cropping and shuffling; mature females in cropping and blocking; and the adult males specialise in allogrooming.

As in the previous experiment with hygienic behaviour, they found that mature females are less likely to disperse if there is work at home. Their strategy makes sense because there are readymade opportunities to gain indirect fitness by staying at home compared to gaining direct fitness by dispersing.

Peter Biedermann, now Chair of Forest Entomology and Protection at the University of Freiburg, Germany, has made another discovery in a more recent study with another species, *Xyleborus affinis*, a sugarcane borer. In this species that was collected from the forests of Louisiana in the US and reared in laboratory test tubes, some of the females who delay or avoid dispersal can also lay eggs in the gallery of their birth, thus sharing reproduction with their mothers.

Taken together, we now have a pretty clear picture of the complex family lives of ambrosia beetles: they are as complex and no less impressive than those of eusocial ants, bees, wasps and termites. Hamilton would surely have been pleased to see the study of his 'funeral feasts' come of age, lend support to his ideas of social evolution and raise many new questions about the mechanisms by which these tiny insects achieve such behavioural complexity and flexibility.

Ambrosia beetles give us added reason (if we ever needed one) to stop wantonly destroying natural habitats and help conserve what is left of biodiversity in general and insect species diversity in particular. Unlike large and charismatic endangered animals, insects present a unique paradox. They are so easy to lose without even realising their disappearance. It is estimated that we may have permanently lost 70 to 80% of insect species in some habitats. And yet, much can be achieved relatively easily.

In his new book *Silent Earth*, Dave Goulson captures this paradox – by painting a stark picture of the ongoing insect apocalypse in the book's first half and provides many workable countermeasures to avert the apocalypse in the second.

Most of us can do precious little to save the tiger or the whale, but every one of us can help save many insects by providing space and suitable environments for local insects. And if we wish to go a step further and study the community under the bark, there is a bark and ambrosia beetle research and outreach community ready to help.

For any Indian aspirant of ambrosia beetle research, there is much inspiration to be had from the early work of Cyril Frederick Cherrington Beeson (1889-1975), an English entomologist and forest conservator who helped develop the Forest Research Institute in Dehradun.

Not long ago, my institute was very pro-green, but their brand of a green environment consisted of growing manicured lawns by purchasing expensive insect-resistant grass and quickly clearing all dead trees and plants, lest they make the lawns look ugly. Let's at least realise that even dead trees can be a paradise for insects and that the family lives of the funeral feasters under the bark can be really beautiful.

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Gadagkar, R., 2021. More Fun Than Fun: Beetles Lead Complex Social Lives in Dead Trees. The Wire Science. URL https://science.thewire.in/the-sciences/beetles-lead-complex-social-lives-in-dead-trees/