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Bionetworks, system biology, and superorganisms

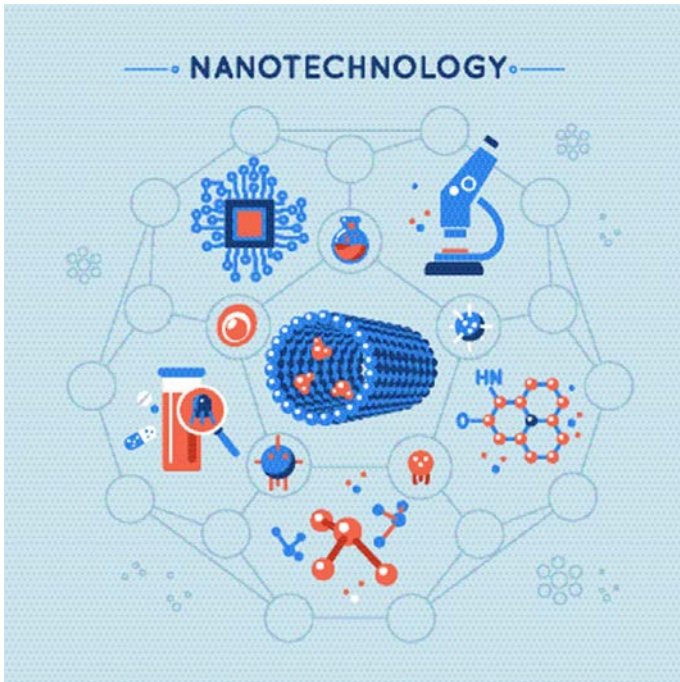


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Looking for new solutions

What we call a “superorganism” is an integrated system involving several types of organisms, able to obtain a final result. The term superorganism was introduced in 2008 by the Human Microbiome Project to consider the human body as made up of both microbial and human cells, being considered as a unique ecosystem (Turnbaugh et al., 2007; Bik et al., 2006). This

approach evidences the relevance of microbiome in human homeostasis, accounting for the trillions of microorganisms from thousands of different species populating every part of the human body. A brave integration between the most primitive and the most advanced cells. Among them, some germs exert a positive effect on health and others are potential pathogens, even in the same population as effect of the environment pressures. This concept is here extended to report the interactions between organisms not necessarily living inside the same body at the same time, and the approach can result related to network of finalized activities, most of them unconscious and endured. Therefore, a common body is not necessary, because the same approach can be applied at an environmental level. In fact, in this case attention must also be focused on the interactions between the involved subjects, using the network model as recently developed in biology. In the case of insect-borne diseases, we can apply the concept of the superorganism in a sequel of bodies and habitats, which must generate a coherent final result.

Insect-borne diseases must be considered as the result of a series of coordinated events, wherein several organisms are involved in a complex situation. This implies some sort of coevolution between pathogen and vector, as part of the superorganism concept. Only the final effect is evident to us, whereas other steps are smartly crepitated or difficult to find. This explains why mankind has spent so much time on trying to understand what was going on, blaming the disease on divine interference or human fault. The concept of “superorganism” is useful not only to understand what is going on in an epidemic episode, but also to understand the role of each organism, and find the Achilles heel that is useful to select an appropriate strategy to control the phenomenon. So far, the approach of “limiting the phenomenon by any kind of wall” still dominates and is absolutely present in the protocols to face so-called emergencies (see the *Xylella* case in [Chapter 5](#)). It is a defense strategy, usually overcome in due course, like any immobilization strategy, and useful only as an early and immediate measure. Insect-borne diseases are not only emergencies but possibly represent daily life, knocking at the door of our future. They are not a novelty, but the recent additional episode of a long story. Someone could say (citing Billie Holyday), “It’s the same old story,” the continuous evolutionary ping-pong between competitive organisms and mankind. However, once again, we should find a way out with the help of science and technology—but we must find this way, and the target is even now moving and very active. Furthermore, it is necessary to consider the presence of several influences affecting seriously the natural tendencies by mankind’s impact.



Mao and the sparrows

So far, the history of governmental measures to avoid the consequences of borne-insect diseases in order to defend and increase population health include plenty of failures and incredible errors. The main idea was to find an easy and rapid solution, usually based on killing the target enemy. In practice it is the usual shortcut to reach a rapid solution, apparently as simple as usually unsuccessful in the reality. In general, the negative effects were much more than the positive ones, when present. This is a point in common between communism, socialism, capitalism, and neocapitalism: the capacity to enact enormous disasters on the environment, presenting them as necessary for the community. There are several clear examples, but one in particular is considered here as evidence. The following case is interesting, at least for those new to it, and is also useful to learn how governments, which are usually accused of lack of action when it comes to environmental problems, can on the contrary be responsible for great perturbations.

Between 1958 and 1962, Mao Zedong, also known as Chairman Mao Tse Tung, was the leader of the Communist Party in China. The external enemies of national communism were defeated and it was time to shift attention to the internal enemies, any kind of enemies. Chairman Mao's first problem was the necessity of producing enough food for the immense population of his country; the second one was how to reinforce his leadership through the recall of the population to massive mobilizations. We have no reports about the advisors and reports that convinced Mao, but there was evidently an error in the information and a misrepresentation of the reality, a typical horrible mixture between stupidity and ignorance, the mother of pointless disasters. Scientists are in such cases stone guests, asked to quantify the entity of the announced tragedy. Thanks to his political superpower and undisputed charisma, Chairman Mao announced and led a giant economic and social movement throughout the country, known as the Great Leap Forward, calling all population to active participation. One of the movement's first initiatives was the Four Pests Campaign, also later passed through the history as the Great Sparrow Campaign or simply the Kill a Sparrow Campaign (Krupar, 2002; Butt and Sajid, 2018). The central idea of the Four Pests Campaign was a move toward public hygiene, identifying four targets considered to be major pests: rats, flies, mosquitoes, and sparrows.

Although very generic from a taxonomic point of view, three of the four pest targets may seem to us reasonable and in clear sympathy with this book, because of their role in spreading plague, typhoid, and the malaria—but what

about sparrows? It is true that birds can be fundamental in the diffusion of insect-borne diseases in the case of viruses such as West Nile fever and St. Louis encephalitis, but these diseases are common in other regions of the world, and the focus on these birds was totally different. Sparrows were included in this list because they consume rice and other cereals from agricultural fields, stealing food essential to Chinese citizens. The Communist Party called people to act together against the pests, focusing on their extermination. The aims were publicized by the efficient capillary propaganda of the Communist Party, through posters illustrating the need for fly swatters, drums, gongs, and even guns as tools in the fight, with the publicized target focused on the need to improve public health. People reacted as expected and took all possible and available measures in order to kill these four types of creature. In the case of sparrows, any sort of means was encouraged: nests were torn apart, eggs broken, hunting trap bird nets were everywhere, and fledglings were killed. Farmers were convinced that killing sparrows would be a good way to protect the local harvest. Furthermore, citizens, scholars, work groups, and government agencies were encouraged to make any kind of noise, with pots, pans, and drums, to scare the sparrows continuously until they fell from the sky from exhaustion. In terms of accomplishing its objectives, the Four Pests Campaign was initially considered a success, with the deaths of 1.5 billion rats, 1 billion sparrows, more than 220 million pounds of flies, and more than 24 million pounds of mosquitoes. However, we must always remember that the real objective was the improvement of public health.

Today, we are used to calling events “fake news.” There was a total error in assigning the role of sparrows in the environment, in particular as an integral part of crop protection. Not only do sparrows eat just grains when possible, but their main food consists of insects, as is the case for most birds. This was demonstrated by scientific reports evidencing the real role of sparrows. In 1959, researchers at China’s Academy of Sciences performed autopsies on several dead sparrows. They discovered that the majority of their stomach contents was made up of insects and not grains, as previously claimed. The result of the campaign was that the insect population in China actually grew exponentially. We must note also that sparrows are the only natural predator of locusts. Locusts eat every plant and are very dangerous, as demonstrated by the biblical plague. The imbalance between predator and prey in the form of sparrow and locust was forcibly broken, obtaining an opposite result. A massive excess of locusts was able to proliferate and swarm, eating the majority of the agricultural production intended for human consumption. In addition,

beside the hundreds of thousands of pounds of grain eaten by the locusts, they were able to profit from conditions of drought, flooding, and other changes due to agricultural policies. Besides rendering the sparrow nearly extinct in China and the reduction of crop production by 15%–70%, the Four Pests Campaign had as a collateral effect the starvation and deaths of humans. When the famine ended, and the equilibrium was partially restored, between 15 million and 36 million individuals had died.

Therefore, when sparrows were saved from the government's insanity, and when the government realized the important role of sparrows in pest control and successful agricultural harvests, another target was implemented. In 1962, when the Great Leap Forward campaign finally ended, this was not the end of the Four Pests Campaign. In 1998, the Chinese government revived a new version of the movement against pests. The campaign against sparrows was over, but the Chinese government replaced the sparrow target with bed bugs. This caused no agricultural damage but did nothing to prevent the Great Famine. Again, posters were seen urging citizens to kill the four pests, this time rats, flies, mosquitoes, and cockroaches. The campaign was a waste of time, considering that people had already been killing these pests before the posters were hung, off course without obtain their extermination.

In this case, as in others presented in many other parts of this book, on one side the butterfly effect it is evident. This effect is well-known, but less known is a corollary of this effect, considering an evaluative differentiation between the species. In the quantum theory interpretation of composition of matter, we must consider the need for an intimate diversity and the consequent difference in the impact. The concept of the central importance of each organism in the habitat (again derived by Darwin) must be in part revised. Some species, named keystone species, may have a more significant impact than others in the habitat equilibria, and attention should be focused on this aspect, since the conservation of all organisms is the ultimate aim. Thus, we have seen the superior impact of the Asian tiger mosquito, and the presence in a population of exceptional individual entities whose increase can change the development of all species and their impact.

This is only one of a plethora of cases evidencing governmental incapacity to identify adequate measures to solve environmental problems without political influences. On the basis of this consideration, what can be done? Wait until the boomerang effect restores the natural order of everything? Meanwhile, stand inert in the face of all the consequent disasters? Is it possible to develop reliable independent scientific models?



Computational reality

The main problem is that so far, everything has been perceived as a comparison between opinions. The real identity of an environmental emergency is usually not an object of sufficient consideration. It is necessary to distinguish the solutions of the environmental problems from political and profitable interferences, which are inevitably typical of human nature. In this confusion, the evidence and indications supplied by science must be absolutely convincing and solid. Nowadays, there is a new fundamental tool that has come out from the environmental cul-de-sac. The aid of an objective point of view is absolutely necessary and can be trusted, with awareness of its limits.

"Can you do Addition?" the White Queen asked. "What's one and one and one and one and one and one and one and one and one and one and one?"

"I don't know," said Alice. "I lost count."

"She can't do Addition," the Red Queen interrupted. "Can you do Subtraction? Take nine from eight."

"Nine from eight I can't, you know," Alice replied very readily: "but –"

"She can't do Subtraction," said the White Queen. "Can you do Division?"

Lewis Carroll, Through the Looking Glass and What Alice Found There (1871)

In the extract above, Alice is in trouble with typical problems of arithmetic—a simple one concerning a calculation that is apparently elementary but beyond the limits of her memory and demonstrating the difficulty of adapting ordinary logic to any kind of calculation. For many years, people were convinced that computers were mainly useful to save them from the difficulties of calculus and would be able only to perform repetitive simple operations. This second point ignited an ongoing debate. Meanwhile, the scenario changed completely and rapidly overcame the limits of a presumed full human monopoly of any form of intelligence. The correct acknowledgment of the existence of another form of intelligence, and therefore the adequate utilization of its potentiality, is the real challenge of the 21st century. However, the reality claims its rights for an independent future, despite the human tentatives to realize an ideal result. Computerized Artificial Intelligence (AI) is fully integrated in scientific activity, as well in ordinary everyday life. However, since the beginning of the computer era, a fundamental debate about the possibility of trusting AI's capacity to help human judgment has been underway. As a confirmation, the story of the evolution of AI is full of uninspected upheavals, as consequences of responses to fundamental questions with spectacular contributions by some of the most talented minds of the last century. The pathway to the AI's achievement was

full of twists and unexpected collateral effects. However, it is possible to coherent in a red thread leading to AI as we know now, such as androids, cyborgs, avatars, and cyclones. The debate on AI is nowadays more present and intriguing than ever. Waiting that the alter ego game furnishes the perfect symbiosis overcoming the Imitation Game, we can travel through the main steps of the AI spectacular outset.

First act: The stone in the pond was launched by David Hilbert at a conference in 1900. The famous German mathematician proposed to the scientific community 23 unsolved problems with the intent to define mathematics logically using the method of formal systems. In this way, the main goal was the developing of a finalistic proof of the consistency of the axioms of arithmetic by demonstration of a series of solutions to the problems. The formalist approach of Hilbert's school intended to develop in a more rigorous way the axioms of arithmetic proposed by Peano, showing the absence of any contradiction inside the system.

Second act: The challenge was accepted by a young and unknown Austro-Hungarian-born, later American, mathematician. A logic arguments enthusiastic, Kurt Friederick Gödel (1906–1978) occupied a central role in the debate. In 1930, Gödel was present in a conference in Königsberg and at the final discussion, in complete but firm quietness he made a complete revolution of the mathematic bases and exposed the demonstration that such a finalistic proof is provably impossible, being auto-referent. One year later, Gödel published his two theorems on incompleteness, when he was 25 years old, 1 year after finishing his doctorate at the University of Vienna (Tieszen, 1992). He stated in the First Incompleteness Theorem that “any effectively generated theory capable of expressing arithmetic cannot be both consistent and particular”—in simple terms, he accused mathematics and logics of working on circular hypotheses, meaning that the whole system suffers from auto-referentiality. The novelty was that Gödel's proposals had an enormous impact, even in mathematics, since probably the most important effect was that the operations of logic deduction can also be assimilated, in their intimate nature, to mathematical operations. In other words, the language of logic in several ways reassembles a particular form of calculation, changing symbols and operations. Gödel has been compared to a barbarian attacking the citadel of mathematics, and in fact his proposals were able to disrupt some precedent axioms and open the way to a new approach. He was quite conscious that the consequences were out of the mathematic themes, involving directly the semantic front, as evidenced in the correspondence between himself and Rudolf Carnap.

The castle of formalism had been seriously attacked, but was still not demolished. Therefore, the initial attempt supporting definitively the axioms of mathematics resulted in a complete crisis of the former system and the generation of a new logical approach. Although it was evident that the axioms of arithmetic were not consistent, however this was not sufficient to open the way to the new paradigm. It was necessary to solve the mother of any discussion in logic: the possibility to have a method able to decide in any situation if a proposition inside a formal system can be considered true or false. The shadow of the Epimenides paradox was still confounding the pathway of human logic.

Third act: The semantic front was at the same time the weakness of the old system and the starting point of the new one. The possibility to exit from the impasse, establishing a defined method, even consisting in a mechanic procedure, was proposed by a young scholar of Cambridge, Alan Mathison Turing (Gandy and Yates, 2001). In the summer of 1936, he published the definitive assault on Hilbert's mechanistic apparatus in a single determinant article consisting of the description of a hypothetic apparatus (Tseuscher, 2005; Soare, 1996). In his "On computable numbers, with an application to the *Entscheidungsproblem*," Turing not only completed the mission of demolishing formalism, but was able to go much further, and this is the central point for our discussion. First, we must consider that Turing was not exactly a mathematician, since he was arguably far more intrigued by chemistry and biology. Therefore, the end of a paradigm inevitably opens the door to other possibilities and a new reality not previously imagined. The paper is based on the description of a hypothetical mechanic apparatus, later named as the Turing machine, in principle able to perform analytically any calculation possible for a human being. The idea was not exactly original, having been already proposed by Euclid, Leibniz, Babbage, and even Hilbert. However, the tendency was negative, and the general idea, as stated by Andrew Hodges, considered that only an incompetent tremendously naïve person could think that mathematics could make a discovery by just rotating a knob on some miraculous machine. Turing essentially was fighting against a prejudice deeply rooted, but he was also attempting to establish the existence of an algorithm able to solve any mathematic problem, which could be the solution of any human controversial by the adequate calculus. In other words, if the solution cannot be found inside the system, one should look outside of the system.

Using his machine, Turing's aim was to demonstrate that beside the classes of numbers and functions computable, there is another class with

numbers and functions not computable, and these cannot be calculated by human beings or by the machine. In other words, the *Entscheidungsproblem* (Problem of Decision) (Turing, 1936) does not have a positive solution, but the Turing machine opened the route to new perspectives. Therefore, the Turing machine was imagined as a method to complete Gödel's deductions, but it was immediately clear that the implications were far beyond the initial scope. This was, beside the practical applications, the reason for the enormous success of the Turing machine. The soul of the Turing Machine is in the possibility of obtaining a total similarity between the logic approach of a computer and of a human mind, through the same semantic approach. The functionality of the machine generated the fundamental difference between hardware and software, causing the birth of the current computers and similar dispositive, shifting the debate from mathematics to the role and future of AI.

The moral of this story is that the effects of an invention can reverberate and be successfully applied in fields far away from the starting research point. The typical effect of application of technology to pure science, often with results totally unexpected and very distant from the starting point of interest. In my laboratory, we call it the Fosbury effect. Dick Fosbury could never have used his revolutionary back-first technique (the "Fosbury flop") in the high jump and won Olympic gold, without breaking his neck, if he had been forced to land on the sand instead of a soft mattress in plastic, recently invented at that time.

In addition, the introduction of vocals in our alphabet is due to the necessity of stonecutters in the ancient Greece to nominate with major precision the names of rich sponsors of their works. In this way, it was not the elaboration of a grammar study or the erudite elaboration of a minister, but the will to exhibit the gratitude by ex voto statues, dedicated to the gods' benevolence, that was at the origin of the complex alphabet. In such a way, the simplicity and utility of our alphabet symbols were considered more adequate for the working process of Turing's machine against other forms of writing, such as Chinese ideograms. An ideogram contains stylized direct information about the form and the nature of the object referred to, but by mixing consonants and vocals you can obtain infinite combinations, as well as with numbers, albeit etymology is lost. It is important to reflect on this point. Language started from the necessity to describe reality and transfer information about it, in a written or phonetic way. Therefore, everything starts from a sensorial perception. Ideograms are the product of visual perception and therefore are designs. Syllabic writing is the result

of an acoustic experience, the conversion of phonemes, sounds, or noises in symbols. That the word A originally was the head of a bull, but it is out of any iconographic value when we use the letter, as well B was a house. The iconographic value of these letters is completely lost as regards their original importance. The alphabet is a series of atomic phonemes, which when combined can give the entirety of the phonetic possibilities, corresponding to parts of reality. In the alphanumeric system, the symbol itself is nothing and therefore can be utilized universally. The negative ontological essence of the alphanumeric symbolism, frozen inside a letter devoid of intrinsic meaning, found the epistasis of its performance in the possible representation of what is considered real and therefore identifiable in physic and metaphysic identification. A computable symbol structurally cannot represent more than nothing, but it is asked to find a precise identity when converted by an algorithm, i.e., consisting of a word inside a phrase or a number useful to express an exact quantity.

This logic was ready to be easily converted into the computer ratio, consisting of nothing more than a further symbolic metamorphosis. During the sequence of metamorphoses of the Occidental language, there was a progressive reduction of the number of symbols and a solution to the phonetic pronunciation problem. The ideographic system is based on thousands of graphemes; the syllabatic one needs 40–200, the consonantic-vocalic 20–30, and the binary one only two, but the latter is still able to represent and interpret reality. Deprived of any visual or phonetic linkage, the symbolism finally acquires its perfect universality necessary to integration. Thus, in AI is a correct evolution of the language system able to be useful to us and to the machine, in the time.



Semantic nihilism and the Turing machine

Among the aims of the Turing paper of 1936, as present in the original version, there was an analogy between its model of machine, named unorganized, and that of a neural network, as elaborated in the same period by [McCulloch and Pitts \(1943\)](#). Once we have accepted the analogy between computer and mankind, as evidenced by the ability to convert reality in symbols, like that of an object, in numbers able to reproduce the exact image of the object, more or less as we do looking at the object (as speculated by Steven Pinker since 2011), we must admit that this is only the simplest part of the problem ([Pinker, 2011, 2016, 2009](#); [Schlinger, 2004](#)).

What about the interpretation of the object behind its form and appearance? There is a limit of complexity in this work of acquiring the reality around? Can a computer with AI give a solution to any problem using the appropriate algorithm or it is something more sophisticated necessary? Pinker's approach is related to the quality of the input, but what about the elaboration, or perhaps the manipulation, of the acquired data as the necessary output? Can everything be converted into numbers or other symbols without loss of information, and the conversion utilized adequately? Essentially, can computerized AI be applied successfully to biological problems? It is possible that the analogy between man and computer is pure fantasy, as stated by Gandy (1988): "There is not suggestion that our brains work like Turing machines." The same postulations of the conceptual Turing machine can be considered as the consequences of pure speculation, like the load-bearing columns of a building whose basement is totally unknown, as evident in the preference about the occidental nature of symbols, and computer and computer are only the appealing facades of the construction, but in any case adequate to obtain an useful result for the topic. In this incompleteness there is also the success of computer machinery (Cooper, 2012).

Although the limits of AI are still unknown and undetermined, being in continuous expansion, the general idea is that a biological process is too complex and variable, but this is a typical irresistible challenge for a scientist, and the Enigma machine is a wonderful example of the enormous potentiality of AI, solving problems and leaving aside any theoretical considerations. However, what you expect from a machine is essentially that it works to satisfy your requirements, regardless of the logical and philosophic problems.

Fourth act: Apparently, the Turing machine is very similar to a typing machine (Fig. 4.1). The device and mode of operation of the Turing machine are easy to imagine and understand, so simple yet so innovative

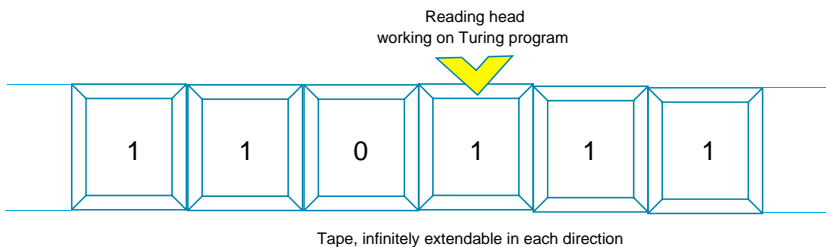


Fig. 4.1 The Turing machine as tape and reading head.

for that time. The heart of the machinery contains a head and a tape. In an idealized computing device, a read/write head (the “scanner”) operates with a paper tape passing through it. The tape is divided into squares, and each square bears a single symbol—in a binary system, 0 or 1, for example. The symbols are rewritten and converted into the machine language. In this way, the tape is also the machine’s general-purpose storage medium and it is able to serve as the vehicle for both input and output and to store the results. Turing’s aim was to show that the tape, and therefore the input inscribed on the tape before the computation, must consist of a finite number of symbols. However, the tape is of unbounded length, given the possibility of unlimited working memory and data storage.

The working memory can therefore store the results of intermediate steps of the computation as well as operate further manipulation in accordance with a programme. The operations consist of altering the head’s internal wiring by means of a plugboard arrangement. The computing consists of inscribing the input on an infinite linear tape (e.g., in binary or decimal code), placing the head over the square containing the leftmost input symbol, and setting the machine in motion. Once the computation is completed, the machine will come to a halt with the head positioned over the square containing the leftmost symbol of the output (or elsewhere if so programmed). The Turing machine can be interpreted like a mechanical transposition of writing and reading done by a human being. The line of the computer screen in Microsoft Word is divided into single spaces, like a child’s squared notebook. The eyes of the writer and its fingers, moving along the line, are the inputs whereas the outputs are the acquisition of data and the delivery of the machine. The writer and the machine are simultaneously flowing back and forth along the track of a well-defined pathway. When the thoughts and the way of thinking of the writer are transferred inside the computer, they are converted into an intangible matter able to be stored and immediately transferred by the computer network, after a deep change of the symbolism. The transfer generates a radical metamorphosis, although the writer is no longer aware of the transformation. Thanks to this manipulation, the ideas can be aggregated with others and converted by algorithms. The simple act of writing, for instance, to enquire about the planetary web system, constitutes the enquire part of the system, modifying in part the system itself and the writer. The litmus test is the simulated experiences in virtual reality, able to convert in part or completely the real world by a computer program. The parallelism computer/computer is the principle of operation in the Turing machine. In the itinerary dictated by the

communication, the idea, generated by the mind, is crystallized in the sound of the phoneme and later in to calligraphy. In this way, the idea can go back finally to the mind (background effect), where it is sedimented as a modified representation of the reality. The representation is crystallized, transliterated, manipulated, and shared through an atomic system of alphabetic or numeric elements, but it seems that something is missing. The debate about the inner significance of the word has a long story connected with its de-somatization. Plato in *Cratylus*, reflecting about the theory of forms and elements, which is the theme of the dialogue, counterpoises the arguments of the sophist Ergomenes, which considered names as superficial and without value, like elements of a society game, and *Cratylus*, evidencing the ancestral nature of names, because “everything has a right name of its own, which comes by nature, and that a name is not whatever people call a thing by agreement, just a piece of their own voice applied to the thing, but that there is a kind of inherent correctness in names, which is the same for all men.” The debate continued for centuries about the enigmatic *lekton* (whether expressible or significant, or simply referring) and has recently been revitalized.

The ticking of the Turing machine in its movement on the tape is the manifestation of the mechanic transposition of the passage of phoneme/writing into a digital version. In the writing and reading acts, there is a fundamental step. In the early days of writing, these acts were prerogatives of an élite: a communication necessary to exchange information and ideas among the élite, or to transfer orders to the population, giving again physical form. During these acts, pronunciation of the words is possible because the written words are transferred as images inside our mind, interfacing with what is already there, deeper and deeper, in an attempt to give physical form and manifest the immaterial, the *pneuma*, the *psyche*, the breath of soul, perhaps in a poetic form. If the writing form is the transaction of voice sounds in visible signs, *semeia anthropines phonées*, silent reading can make an immediate link to the eyes, establishing a path to the beginning. The art of thinking can be fully expressed and achieved by intelligent machines. Symbols, signs, and even images and sensations can be easily converted, stored, and shared independently by the chosen language. Again, parallelism emerges: electrons moving in a neural network and electrons running in software. The optimization of this process has a cost, like any evolutionary jump. Trusting a powerful tool like AI generates dependence. The clear general difference that has emerged during the last decade is the ability and confidence in the utilization of PCs and cellular phones. In a world running toward the dominance of apps, this is a line of demarcation. When your money is only a number inside

a file and you must use an app to move it, if you are not able to, you cannot ask for help from the bank because it does not physically exist; there are no agencies, no bankers, no one to talk to and explain your case to—only an app. Right or wrong, this is the present and the future we must consider.

There is an ancestral resistance for novelties, in particular if they are difficult to understand and appropriate. Many people are dedicated to using AI, because it eases their life, but they are diffident about what they do not understand. When mathematics are involved, caution becomes suspicion and mistrust. This attitude involves also educated people and most politicians, and it can result in violent reactions. I am deeply convinced that the similarity between the tragic ends of Turing and Palamedes is not coincidence. Both crystalline heroes and models of pure intelligence, creators of novelties, inventors of numbers and symbols and of their significance and utilization. Both envied and persecuted until their dramatic ends. Both scapegoats sacrificed in favor of the dominance of models produced by perverse *callidus* Ulixes.

Once accepted the compatibility between the computer, the intelligent machine, and the computer, the model and creator (or vice versa), based on the same computational approach, the next step is the integration between the enormous computational capacity of the computer and the infinite intuition of man. The idea is that the sequence reality/symbol/computation needs further steps to avoid the limits of simple representation. This is possible thanks to the dual nature of the computer, the behavioristic one based on the direct relation between external stimulus and compartmental replay in a contest essentially deterministic and operationistic, similar to the computer operability, and the second mainly cognitive and representative of the inputs of the previous stage. The two states are in continuous interdependence in order to obtain the final result, consisting of the consciousness of reality and the decision about the adequate comportment. To maximize the final result, correct acquisition of as much environmental data as possible is preferred. However, among the condition for a useful interaction between computer and computer, the communality of symbols and arrangement of data in the best comprehensible manner are key to integration—in other words, whether the evolved form of the Turing machine can become bio-logic or remain bio-graphic. In this operation, the data are connected in a logic representation, but usually after a simplification, based on the determination of hierarchy of interest: a screening of what is of primary or secondary importance. The time is right for computational and modeling bioscience.

This long dissertation about AI is a necessary preamble to the next part of the chapter. About the digitalization we live a controversial experience. Most people are convinced of their absolute control over the computer, since it is possible to switch off the machine at any moment, but dependence is another matter. However, when we are not online, we are still likely to be connected and part of the net system somewhere.

The similarity of the computer and computer is not in the symbolism, but in the segmentation of the tape. In other words, the key is in the empty space separating the symbols. Reality is based on continuity, wherein the present does not exist, being a hypothetical passage to the future from the past. The Turing machine, as any alphanumerical system does, works in a discrete sequence. This is the original sin still marking the difficulty of any human in his tentative to represent and interpret the present as a continuous flow of the reality.

The alternative possibility of AI to nab the complexity of biological systems is if the biological reality is like a giant chess game, wherein each organism plays its game maximizing its capacities but according to rigid rules, or the flexibility and variability are so high that any prediction is impossible, like in the croquet game of Alice in Wonderland where the balls were live hedgehogs, the mallets live flamingos, and “the players all played at once without waiting for turns, quarrelling all the while, and fighting for the hedgehogs.” The scientist may feel very uneasy, like Alice managing her flamingo. Therefore, AI is necessary—first, because general physical–chemical rules exist and living organisms cannot escape; second, because in the habitat all players play at once but rationally influence each other; and third, because systems can be simplified but variants need AI to be calculated as well human sensitivity and intelligence to be understood and verified.

AI is a powerful tool, but it also possesses independent capacities, which is both a problem and an opportunity for any technological advancement. So far the predictivity of AI in outbreaks was practically null, despite all the progress and tentatives. As evidenced clearly by the COVID-19 episode and the debacle of algorithms produced to interpretate epidemic trends, as evidenced by the GFT (Google Flu Trends) program about the trend of stagional flu in the world. In terms of the themes of this book, so far the help of AI in solving insect-born disease events was also very limited with regard of migration and boom of certain vectors. In Zika outbreak time, there was a titanic effort, to consider the simulation of a billion of people and six billions of mosquitoes utilizing 7500 computer only to evidence the pathway to the Zika virus from 2014 to 2016. However, so far these projects were not able

to afford more results to understand the past than to predict the future. As for climate change, most of politicians, beyond the official crocodile tears, are doing nothing of practical effect, and several of the them still wave the flag of the typical propaganda that “the sky is always blue”—until the next catastrophe. However, scientists must continue with their research and their computerized models, and, very importantly, they have to work hard in transferring their knowledge to the ordinary people, who can assume responsibility for their own destiny. The solution is not to “ask the computer” and abdicate all personal mental judgment, but to be aware of the need for independent information and evaluation. Meanwhile, reassuring signs are coming from the scientific side.

The first attempt has been made on the cell, known as system biology (or biology of systems) (Breitling, 2010). Further advances were obtained by an exponential development in parallel models and highly advanced scientific devices. Over the past 30 years, medical research analytical technologies have arrived at the point where most (if not all) key molecular determinants deemed to affect human conditions and diseases can be scrutinized in detail. The final aim is that the utilization of these technologies, and others such as quantitative polymerase chain reaction (qPCR) and next generation sequencing, enables extracting information from complex datasets to obtain disease models to be developed and verified by wet-lab testing.

The study of biological systems, like those present in living organisms, are based on the following axioms: (a) a biological behavior cannot be reduced to the linear sum of their parts’ functions, following a holistic approach instead of the more traditional reductionistic one; (b) the complexity of the system under study is not a limit but a positive aspect; (c) technology and computation are integrated in the approach to the elaboration of the solution; (d) data are obtained mainly by transcriptomics, metabolomics, and proteomics through high-throughput techniques, and dedicated to the construction and validation of models, the model being the real result of the process; and (e) contribute to stratified the so far dominating medicine en route to an approach of personalized health care.

In practice, system biology is simply the application of dynamical complex system concepts to the molecular metabolism using a sophisticated computerized approach, named the computational model (Baitaluk, 2009; Tavassoly et al., 2018; Zou and Laubichler, 2018). The theory of self-organized systems means the possibility to understand the internal logic of life, through the manifestations of cellular metabolism (Schrodinger, 1944). According to Sauer et al. (2007): “the pluralism of causes and effects in biological networks is better addressed by observing, through quantitative

measures, multiple components simultaneously and by rigorous data integration with mathematical models.” Among the aims of system biology, predictivity is the natural central result of all the process, generated by the integration of the available data, designing principles of biological circuits in the organic network. Systems medicine (Ayers and Day, 2015) is probably the most important application of system biology, serving to identify clinically important molecular targets for diagnostic and therapeutic measures, including the discovery of new pharmaceutical drugs for fighting, or at least controlling, the resistance phenomenon in insect-borne diseases.

Today, the cost of experiments, trials, claims, and marketing is a serious limit for the development of new medical drugs (Moore et al., 2018; Sinha et al., 2018). In 2018, a team including researchers from Johns Hopkins Bloomberg School of Public Health reported in *JAMA Internal Medicine* that clinical trials that are considered necessary to support the US FDA approvals of new drugs have a median cost of \$19 million. However, the study suggests that these costs of the “pivotal” clinical trials contribute only modestly to the overall costs of developing new drugs, because it is necessary to consider all the previous investigation and study. Therefore, the \$19 million median cost represents less than 1% of the average total cost of developing a new drug. This cost of pivotal drug trials is variable in the different classes of drugs, but in recent years it has been estimated at between \$2 billion and \$3 billion, including \$2 million for a four-patient trial of a treatment for a rare metabolic disorder, up to a mean cost of \$157 million in cardiovascular drugs, and just \$21 million in endocrine and metabolic disease patients. The major cost is \$347 million for a large study of a heart-failure drug. Although the market of an efficient drug in these diseases is enormous, it is evident that such costs, although totally justified by the need of health security, are killing off the pharmaceutical industry, in particular for antimicrobials. Although several studies are underway to lower costs and develop better trials (Moore et al., 2018), the main unsolved problem, as already mentioned, is multidrug resistance. In a few years, microbes can find a way to develop resistance, before the necessary return on the investment in terms of money and human resources. Again, computer aid can furnish at least partial solutions.

The final aim of system biology is a reliable model of the cellular system, wherein it would be possible to test the biological activity, avoiding the usual current tests based on living materials, i.e., cells, tissues, organisms, and consequent ethical and economic problems. In other words, a simulation approach, similar to that used for instance in the training of airplane pilots, involving a careful and methodical examination of all simultaneous molecular interactions at the occurring cellular levels. In this way, the costs

of clinical tests could be avoided or decreased, as could the sacrifice of animals. However, so far, to obtain a reliable simulation in perfect synthesis with the Turing machine, a process of elimination of the details considered nonessential seems necessary, based on suppression of redundant and enucleation of invariant factors in search of the “essential character” of the living system under examination. This is probably the main limit and the most delicate aspect of any computerized apparatus when applied to a dynamic system. Once the coherence in functionality and compartmental features between computer and computer for input, storage, and utilization of the database is accepted, who takes responsibility for choices? Apparently, the computer possesses the necessary ratio and sensibility, but his or her capacity to manage the amount of data is limited and the objective capacity of analysis could be influenced by emotional factors. In contrast, the computer could be objective and respect the necessary scientific approach. In reality, all these considerations are wasting time: driving forces have been acting since the birth of the Computer Age for an integration between computer and computer, a hybrid naturally born and already affirmed in human society. The reality is that we cannot leave out the AI influence to impose the pathway of the rational use of AI versus the chaotic dangers of “big data” and G^{nth} in every type of our activity, in particular for the predictive ones.

The continued goal of AI is to trust the capacity of enormously complex machines to examine situations and produce reliable models, but they are ultimately machines, and to do this is possible because human behavior is considered intrinsically mechanic and computational just as that of intelligent machines is. Beside the large debate on the righteousness of this assertion, it is necessary to repeat that this assumption is becoming (or is already) ordinary and scientific reality. Computer and computer (in the original version is female) are still not married, but they are a couple in facts. On the other side, it is conceivable that at least in future the computational model will be able to perform a reliable and generally accepted simulation of a living system.

A cell, like an organism, is the result of an organized functional network of individual components. The aim of this organization is the correct and useful connection with the external conditions. This definition can be simply utilized to describe the environmental equilibria. The model simulation in this case changes the actors and the place, but in principle the approach can be similar. Therefore, the utilization of a computerized approach to produce reliable models is conceivable also in the case of the determination of

factors influencing environmental changes and their effects. The main obstacle to realizing such models is not the complexity of the system, but the utility of such effort. Nowadays, there is a very low probability that such results could be the objective basis of a government policy on management of a habitat. However, it is the duty of science to continue in this direction. In this way, there is a chance of understanding what is going on in our changing world. Thus, in insect-borne diseases we fully understand the environmental abiotic factors affecting the migration of vectors or the pathway of emerging strains with genetic trait changes, and these data can be interconnected with the behavior of the vector and the biotic effects to obtain a model evidencing the future, such as the time and the possible areas of diffusion of the disease. Any kind of measure and intervention by governments and related agencies should therefore be undertaken considering carefully the results present in the models instead of the pressures of private or political interests.

While waiting for advancements in AI, there are other fronts offered by technology; in particular, general attention is focused on the innovations offered by nanotechnology, whose applications can be very important also for future control of insect-borne diseases. In particular, we are interested in the biological applications of nanotechnology, considering the potential environmental effects.



Nanobiotechnology

In this chapter, we are attempting to explore the last frontier of technology and its fundamental role in future control of insect-borne diseases. The argument is recent and still taking its first steps, but its potentiality cannot be underestimated. The relation between nanotechnology and insect-borne diseases control is already known, although so far it is limited to the experimental phase. Perspectives are concentrated on the control of the vectors, focusing on natural insecticide and the increase of activity obtained by the stabilization and protection of the active constituents (Mehlhorn, 2016).

In particular, I was involved in several investigations on the production of green nanoparticles, participating in research by a group led by Prof. K. Murugan (Thiruvalluvar University). The aim of the project was to check the activity of green nanoparticles on insect-borne disease vectors, but also the possibility to utilize different materials and determine the impact on the environment, in particular for predators that are the natural controllers of

preparasites' diffusion. This approach was totally in accordance with my belief in research born from integration of different scientific experts and the need for useful integration between laboratory work and field experiments, as well as the persuasion of the utility of the investigation. The control of insect vectors is the cornerstone of exploration on environment equilibria and the interactions inside the habitat of several protagonists. The focus on the utilization of novel technological tools is clearly important for the future.

Technology consists of the application of scientific knowledge to solving challenges and practical aims of human life, usually related to changes of conditions in favor of human necessity. As slaves of Prometheus's prophecy and emulators of Palamedes, we trust technology to solve the problems of survival and maintain supremacy by advancing the utilization of available materials. Science and technology are interdependent but distinct, and both are consequences of innovation. In fact, although most people consider technology to be a practical and efficient application of scientific discoveries, the converse impact of technology on science opens up novel scientific questions and leads to new and more difficult arguments, enriching and reorienting the agenda of science. Thanks to technology, the full range of science discovery can find allocation and be addressed in an efficient and timely manner.

Let us consider another book. Probably the first convincing explanation of the potentiality of nanotechnology is contained in a short book written in 1944 by Erwin Schrödinger, one of the fathers of quantum theory, titled *What is Life?* (Schrödinger, 1944). The book is a compendium of a series of lectures given at King's College, Cambridge (UK) a few years previously. As evidenced by the title, the book is focused on questions typical of biology, but answers were explained by quantum theory. For instance, it contains an exact description of the molecular nature of DNA, 10 years before its discovery, as "a gene—or perhaps the whole chromosome fibre—to be an aperiodic solid." An aperiodic solid means a chemical compound made by minor molecules assembled in an unrepeated way.

The book starts with the question: "Why are atoms so small?" The answer, in a typical quantum style, is that in this way each atom is free to exert different contents of energy, without interfering with the total macroscopic result. Therefore: "If we were organisms so sensitive that a single atom, or even a few atoms, could make a perceptible impression on our senses—Heavens, what would life be like!" A similar consideration can be applied to cells, or to snow crystals. In this way, variability and

constancy can coexist, without consequences about the confidence in general states of the matter. In other words, the physical laws are the same everywhere, but the mechanics of each phenomenon are not the same and are dependent on the scale.

The meter as a measure is familiar to us because it is closely related to the size of our bodies and to the reality expressed by our senses, but there are other realities, expressed by other units: the limit of our sensibility is 0.001 (10^{-3} m), the one concerning the cells is based on 0.000001 meter (10^{-6} m), and that of atoms and molecules is called the nanoscale and is based on 0.00000001–0.0000000001 (10^{-9} m) meters. In a single grain of sand, there are thousands of millions of atoms, all of them individually different and arranged in different ways. An atom is too small to be seen with any kind of instrument, and its presence can be deduced only indirectly with a special experiment and extreme difficulty. In atomic physics, it is necessary to use as a unit the so-called Angstrom (\AA), which is the $10^{10\text{th}}$ part of a meter, avoiding the decimal notation of 0.0000000001 m, since atomic diameters range between 1 and 2\AA . The atomic world is impenetrable to our senses, but it influences our activity. Beyond this scale, we find the enigmatic level of quarks, where only mathematics and imagination are able to enter, and where something still not clearly understood, like strings, is acting. On the basis of these considerations, all of reality can be depicted as a series of concentric interacting worlds, wherein we can enter and interfere positively only by knowing and respecting the rules of each world. This is important also for the messages contained in this book. The world experimented on by a pathogen organism, like a bacterium (less than $1\text{ }\mu\text{m}=0.000001$), and even more by a virus (20–500 nm), is nearer to the nanoscale than our one; however, the parasite is obliged to interact with the world where we use to live and the solution is inserted into the intermediate cell world.

An insect-borne disease is the result of smart integration of concentric inclusive levels of reality, including also the giant environmental one, with its biotic and abiotic interferences. The knowledge of the homeostatic strategy of a parasite is crucial to understand and select the method to control it efficiently. The approach starts imaging to live in the world of the parasite or the vector, never forgetting that they make all their efforts to be an integrated part of our concentric world: the environment where we exist as a body made by cells wherein molecules act to make life. In nanotechnology, we work starting from the atomic world to influence all other states of matter. The last one can now be explored and influenced, although only

partially, because of its intrinsic difficulties to interfere, but with awareness of its fundamental role. Previously, the molecular nanoscaled world has been out of our direct influence, but nowadays this limit can be overcome.

Let us consider now the problem of biological barriers. Most living organisms, such as bacteria, fungi, and plants, possess a cell wall; even virus have a robust envelope. However, it is absent in animals and the Protista, their precursors. This is the result of an evolutionary step. The cell wall ensures protection and individuality, but it is a source of difficulty for collaboration in the cellular network of the same tissue, and confers separation between the cytoplasm and the environment. Thanks to the absence of the cell wall, communication between tissue cells is allowed and preferred, and it is possible to run 100m in less than 10' or fly with the fantasy, but our cells are also more penetrable to parasites and other intruders. Arthropods are an exception, as they have protection in the form of an exoskeleton, which covers the whole body. This is necessarily a compromise between efficiency and protection. More protection means more separation and difficulties in the exchange of food and energy. An excess of reinforcement of the cell wall implicates the death of the cell, such as in the lignified vessels of shrubs and trees. There are several mechanisms for input and output, facilitated like osmosis, or active or passive transport, but these need energy. To avoid this waste of precious energy, in cell walls there are micro-holes that can be used as open doors for little molecules, such as water, that are able to seep through and enter the cell. Therefore, they are the simplest way to penetrate a bacterial cell. If we have a chemical weapon to kill the parasite, enabling the parasite to work, it is necessary to overcome the cell wall protection, and the micro-holes can be utilized for this purpose. Furthermore, the active substance must be protected during its trip until it reaches the cytoplasm. Until the organic substance is inside a cell, its integrity is usually assured, but outside, the instability is hiding. In particular, in the case of natural products, they are quite stable inside the cell, but they are rapidly oxidized and degraded outside.

Therefore, the key argument in nanotechnology is dimension. To have an effect, the active molecule must enter the cell, but there are protections. If the target is a microbe or a virus, there is a solid barrier constituted by an envelope to overcome; if the target is an insect, the exoskeleton preserves it from invaders. The nanoparticle must be under 50 nm diameter to utilize the nanoholes of the cell wall. In its journey, the active substance is protected inside the nanoparticle. Once that the nanoparticle is inside, its content should be able to realize the toxic effect against the pathogen, like Trojan

horses containing the weapon against the responsible of the disease. However, it should be noted that empty nanoparticles also generate some perturbations in the target cell. The cell metabolism perceives the presence of the alien intruders and reacts in consequence. Therefore, it is necessary to check the mechanism of action to maximize the toxicity.

Recently, the ability to change the point of view reaching the nanoscale gave impressive results and applications, which is likely to be fundamental in the future of science and technology. Nanoscience is a multidisciplinary field based on the design and engineering of functional systems at the nanoscale. Nanotechnologies have the potential to revolutionize a wide array of biological applications, including drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, tissue engineering, and pest management. However, probably the most important impacts must be expected in the habitat, due to its unique ability to interact at any level of the scale, from micro to macro, in magnificent continuity and efficiency.

The first step is focused on synthesis, characterization, and use of materials and devices that could have new properties and functions based mainly on their small size. The creation of nanomaterials is based on selection and assemblage of nanoparticles. The self-assembly is an automatic process, resulting in a geometric architecture obtained thanks to the reduced dimension and the intrinsic properties of the nanoparticle, including geometry and surface properties. Nanoparticles are designed to auto-arrange spontaneously in clusters or greater ordinated structures. Nanoparticles are collections of atoms arranged and bonded together with a structural radius of <100 nm, used as key fundamental components in the fabrication of a nanostructure, whose physical and chemical properties are dependent on the types and amounts of atoms in the material. Nanoparticles are selected on the basis of size, shape, large surface-to-volume ratio, absorption and surface functionalization, and utilized material. Nanotechnology acts selectively at an atomic/molecular level. Aims of nanoscience are related to the fabrication of cheap and more stable eco-friendly, easy-to-use marketed products, which are able to interact in atomic systems. Nanoparticles can be used directly to obtain new materials or to insert inside active principles, like in microencapsulation of drugs in pharmaceutical chemistry or the formation of films in intelligent packaging. The choice of appropriate materials is a prerequisite of any nanotechnological application.

In the last decades, noble metal nanoparticles have been preferred for synthesis of nanomaterials. Metallic nanoparticles are mostly obtained using noble metals, such as gold, silver, platinum, and lead. These metals are

necessary to join together the ingredients of the nanoparticles and obtain their arrangement in self-assembly. Among metal nanomaterials, silver nanoparticles gained interest in bio-research due to their application and properties. Vast applications of silver nanoparticles include size-dependent interaction with HIV-1, bacteria, microorganisms, etc. The chemical reduction of aqueous solution of silver nitrate is one of the most widely used methods for the synthesis of silver nanoparticles.

However, redox synthesis methods generally use hazardous chemicals as reducing agents or require significant energy input. The consequent risk of pollution must be considered. There is thus a growing interest in the use of environmentally safer “green” reducing agents. Nanobiotechnology mimics natural processes. The self-assembly is typical of production of final 3D conformers of the macromolecules and the basis of supramolecular chemistry. Nanomaterial is obtained by self-assembly into ordered layers, whose structure is based on electrostatic forces, like hydrogen bonding, polar and dipolar interaction, hydrophilic or hydrophobic interactions, surface tension, and others, by analogy to the self-assembly of biopolymers, like those present in membranes, vesicles, nucleic acids, polysaccharides, and proteins.

Nanobiotechnology, also named green nanotechnology, is focused on interaction in biologic systems using eco-friendly and safe materials, which means novel and cheap bioreducing agents for eco-friendly nanosynthetical routes. Nanobiotechnology is a new and growing science, focused on eco-friendly green-mediated synthesis of nanoparticles. It is a fast-growing research in the field of nanotechnology with relevant applications. This technology has already been used in several areas of science, such as chemistry, biology, physics, materials science, and engineering, and applied for innovative solutions in drug delivery, diagnostics, gene delivery, and tissue engineering. Nanobiotechnology research in principle can be applied in any field and opens newer avenues for unraveling a wide array of applications in production of biomedical sensors, antimicrobials, catalysts, electronics, optical fibers, agricultural products, bio-labeling, and other items.

Nanoparticles can be classified on the basis of their chemical composition, divided mainly into metal and/or organic constituents. In several cases, the materials can be used at the same time, since nanocrystals of gold, silver, and their alloys have been synthesized with the assistance of organic materials of different types. In insect-borne diseases, nanobiotechnology has the potential to revolutionize the agricultural and food industry with novel tools that enhance the ability of humans and plants to confront the new challenges successfully. The plant-mediated fabrication of nanoparticles is advantageous

over chemical and physical methods, since it is cheap, single-step, biodegradable, and environmentally friendly. Furthermore, living organisms can also be used, since microbes are used in nanotechnology for producing nanoparticles and current green synthesis has shown that environmentally friendly and renewable sources of fungi can be used as effective reducing agents for the synthesis of silver nanoparticles. Green nanotechnology synthesis also comprehends plant-mediated biosynthesis of metal nanoparticles. This nanotechnology is advantageous over other chemical and physical methods, as it is cheap, single-step, does not require high pressure, is biodegradable, saves energy and temperature, and avoids the use of highly toxic chemicals, also in field conditions.

The aim of green nanotechnology is focused on the production of new products with high selected activity and ecofriendly properties. Plant-mediated fabrication of nanoparticles is advantageous over chemical and physical methods, since it is cheap, single-step, and environmental care. As a matter of fact, the impact of long-term exposure to low, highly dispersed doses of nanoparticles on ecosystems cannot be determined easily. Among the aims of green nanotechnology is the utilization of organic materials, avoiding or limiting the utilization of metals or potentially toxic materials in the manufacture of nanoparticles (Govindarajan et al., 2016). Several materials have been successfully used to obtain green nanoparticles, developing efficient and rapid extracellular syntheses of silver and gold nanoparticles, which showed excellent mosquitocidal properties (Murugan et al., 2015d).

On the other hand, nanobiotechnology can be used to improve our understanding of the biology of vectors and parasites, as well as developing improved systems for monitoring environmental conditions and enhancing the ability of plants and other hosts to resist microorganism attacks. Therefore, the goal is not the production of further lethal weapons against the target enemy, but considering several aspects of the action. Nanocrystals of gold and silver, and their alloys, have been synthesized with the assistance of various bacteria as well as the environmentally friendly and renewable sources of fungi used as effective reducing agents for the synthesis of silver nanoparticles. Plant-mediated biosynthesis of nanoparticles may be advantageous as successful alternatives to classic chemical and physical methods and in pest management and parasitology.

Among the experimental results on the basis of appropriate utilization of nanoparticles obtained through the green synthesis route, the necessary evidences of the insert and presence of the active ingredients by biophysical characterization. Nanoparticles can be obtained independently from the

addition of the selected active ingredient. The best solution to this key issue is to adopt a mix of experimental approaches, each confirming the effective internal presence of the organic material. The morphology can be determined by transmission electron microscopy, showing spherical shapes, with an average size of 3–18 nm. X-ray powder diffraction can highlight the crystalline nature of the nanoparticles, ultraviolet-visible absorption spectroscopy is used to monitor their synthesis, and energy dispersive spectroscopy is used to confirm the presence of elemental elements, such as silver or gold. Finally, Fourier transform infrared spectroscopy shows the main reducing groups from the organic content. The last analysis is the most important, showing absorption bands typical of secondary natural products, such as OH or CH or CO stretching. However, my opinion is that IR evidence is quite general and not sufficiently specific to each plant ingredient. Waiting for help from the advancements of technology in analytical chemistry, a solution could be to obtain an IR fingerprint characterizing the plant extract to be compared with that obtained from the nanoparticles, as already obtained by high-performance thin-layer chromatography (HPTLC), as well as referring to information obtained by other analyses.

In insect-borne diseases, nanoparticles possess peculiar toxicity mechanisms, due to surface modification, and they are synthesized to perform selected biological activities. In particular, several reports are present in the literature concerning the potential antibacterial, antifungal, and antiplasmodial and mosquitocidal properties of silver and gold nanoparticles containing natural materials (Suresh et al., 2015; Sujitha et al., 2015). Silver and gold nanoparticles synthesized using *Chrysosporium tropicum* became active against the *Aedes aegypti* larvae; the larvicidal activity of silver nanoparticles synthesized using *Pergularia daemia* latex was screened against the *Ae. aegypti*; and silver nanoparticles synthesized using *Feronia elephantum* plant leaf extract showed adulticide activity against *Anopheles stephensi*, *Ae. aegypti*, and *Culex quinquefasciatus* (Murugan et al., 2016a). Larvicidal and antimicrobial activity of silver nanoparticles synthesized using marine fluorescent pseudomonads was positively tested against various human pathogenic bacteria, such as *Aspergillus foetidus*, and fungal pathogens of plants.

The choice of material is key, considering the large range of useful species and the relative kind of physico-chemical properties (Subramaniam et al., 2015, 2017). In particular, fungi can produce significant amounts of nanoparticles because they can secrete larger amounts of proteins, which directly translate to higher productivity of nanoparticles. The mechanism of silver nanoparticle production by fungi is based on the

following steps: trapping of Ag^+ ions at the surface of the fungal cells and the subsequent reduction of the silver ions by the enzymes present in the fungal system.

Biosynthesis of nanoparticles using biomass was used as a profitable tool, but again the properties of the materials must be considered carefully. In this case, the material needs time to act, and this goes against their natural degradation. In contrast, for the same reasons, natural substances are eco-friendly and must be preferred to synthetic products. It is necessary to obtain an equilibrium between these two opposite strains—a typical situation that needs a technological answer. In recent years, a growing number of plant-borne compounds have been proposed for efficient and rapid extracellular synthesis of metal nanoparticles effective against mosquitoes at very low doses (1–30 ppm).



Making green nanoparticles

Nanobiotechnology is the last frontier for utilizing natural products. Nanotechnology has gained popularity in recent years due to its application in various fields. Nanoemulsion consists in kinetically stable droplets prepared using stable particles of oil and water, and requires lower surfactant concentrations against other products based on microemulsion. These characters are suitable for drug delivery in consideration of the smaller droplet size, larger surface area, and lower surface than the most common other products, based on micro and not nanoscale. It contains antimicrobial activity against bacteria, fungi, and virus.

Other considerations in research on new green nanotechnology approaches lead to choices of material to use in the production of nanoparticles owing to possible environmental effects. Nanotechnology can be important for future treatment of the human habitat. The quality of environment in which we live is a real challenge for everyone. People in towns may worry about air pollution due to chemical agents, while people in rural areas may be more concerned about water and its contamination by microorganisms. Among the most relevant aspects of human evolution, we must consider the progressive tendency to utilize very pure water for alimentation. We need large quantities of pure water, since every 20–22 days, the water in our body should be replenished. However, if urban populations can rely on efficient methods of purification, in rural places pathogens and parasites can pollute waters and constitute a severe plague, especially in

developing countries. The consequences of lack of access to safe drinking water are significant. Intestinal infection causes diarrheal diseases and affects about 1.6 million people globally (about 90% of whom are children), including cholera. A total of 133 million are suffering intestinal helminthes infection, including 1.5 registered cases of hepatitis. The bacterium *Chlamydia trachomatis* causes active eye infections in more than 80 million people and can induce trachoma in about 500 million. The treatment for hygienizing water is usually achieved by addition of chemical coagulants and chlorine, as effective bactericide agents. An alternative green tool to conventional chemical treatments relies on the utilization of plants and plant extracts to clarify and purify water. However, two important points must be considered: the choice of the plant material and the utilization method. *Datura metel*-synthesized silver nanoparticles were utilized to magnify predation of dragonfly nymphs against the malaria vector *Anopheles stephensi* (Murugan et al., 2015d).

In addition to plants of several botanical families, natural raw materials of different origins were used to synthesize nanoparticles utilizing marine organisms, such as seagrass (Mahyoub et al., 2017), and spongweed (Murugan et al., 2016a,b,c,e,f,g,h), and also fern, chitosan, earthworm (Jaganathan et al., 2016), and others, and their activity against vectors was tested successfully.

In a 2015 paper (Dinesh et al., 2015), the plant *Aloe vera* was studied with green-synthesized silver nanoparticles to obtain mosquitocidal and antibacterial activity in field conditions. The genus *Aloe* comprises about 500 species, most of them present in South Africa, but some species are distributed almost globally, either for their beauty as ornamental plants or for their medical and cosmetic benefits. Among the *Aloe* species, the most important is *A. vera*, for its more than 200 active constituents and multiple utilizations as an extract or gel. The medicinal extract, containing anthraquinones, has a long medicinal tradition as a laxative, whereas the gel is a cicatrizing and a skin protective agent that is currently widely utilized. The reported activities of the gel include strong antimicrobial properties against bacteria, fungi, viruses, and yeasts. It is rich in hydrocolloids, since *Aloe* species are succulent plants, adapted to live in arid and hot climates. As other succulent plants do, they survive thanks to the water stored in the mesophyll inside a special tissue, named aquifer parenchyma. To perform this job, this tissue contains mainly hydrocolloids, which are complex polysaccharides, made by a polymerization of an uneven pool of sugars. The main activity of hydrocolloids, as the name suggests, is to hold on to the water molecules. These molecules are polyols and thanks to the large

presence of hydroxyls, they are able to produce a great quantity of H-bonding with the water molecules. Hydrocolloids change the lipophobic nature of water, interacting with the intimate network of water clusters and giving rise to a sort of “quarter state of water,” although this is unstable. They act as a bridge between the water and fats, giving rise to a coexistence between polar and nonpolar compounds. Thanks to hydrocolloids, we have “miracle” products such as soap bubbles, cosmetic creams, mayonnaise, ice-creams, and many more that are consumed in everyday life. Hydrocolloids have very important biological properties, including also toxic effects on aquatic organisms, directly on the target or changing the environmental parameters.

The green-synthesized silver nanoparticles, containing a cell-free aqueous leaf extract of *A. vera*, became toxic to larvae (I–IV instar) and pupae of the malaria vector *An. stephensi* in vitro and in the field, and showed antibacterial properties against *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Salmonella typhi*. In addition, the efficiency of the micro-emulsion, prepared using *A. vera* extract and silver treatment in water treatment, was reported. The water quality was tested by analysis of parameters, such as color, turbidity, and pH, and analyzed over the different water reservoirs, acting as breeding sites of mosquitoes in pre- and post-treatment phases. The results obtained indicate the possible utilization by uniform dispersion to clean water from parasites and bacterial pathogens.

Among the plant materials we utilized in nanotechnology, fern-synthesized nanoparticles were utilized in the fight against malaria. The obtained silver nanoparticles of *Pteridium aquilinum* leaf extract were analyzed by LC/MS analysis and they showed high mosquitocidal activity (Panneerselvam et al., 2016). In another study, the potentiality of fern-synthesized silver nanocrystals was studied as a possible new class of mosquito oviposition deterrents (Rajaganesh et al., 2015).

Another research front was the experimental search for very unusual materials. Again attention had to be focused on our ancestors and their incredible properties, lost during the evolution of complex organisms. The story of the discovery of magnetotactic bacteria is an example of what was and still is going on in academic scenarios. These wonderful creatures were first discovered in 1963 by Salvatore Bellini at the University of Pavia, Italy, and rediscovered in 1975 by Richard Blakemore. It is relevant to report the story of this rediscovery. Bellini, when observing under his microscope a group of bacteria, noticed their evident orientation in a unique direction. Thus, these bacteria, instead of being statically disposed in a circle, appeared well-ordered in a single queue, like soldiers or people waiting for

the bus. Nowadays, we believe that his alignment aided these organisms in reaching regions of optimal oxygen concentration. His explanation was that these microorganisms moved according to the direction of the North Pole, just like the needle of a compass, and, considering the phenomenon to be a consequence of magnetism, called them “magnetosensitive bacteria.” As was usual in European universities at the time, he communicated the results of his experience in Italian in a local academic scientific journal, the *Istituto di Microbiologia*. I well remember that at the beginning of my career, we could access only short summaries in English of research in Russian or in Chinese, published in the official journal of a well-known institution. Thus Bellini’s discoveries remained practically unconsidered until they were brought to the attention of Richard Frankel in 2007. After Frankel’s translation and its publication in the *Chinese Journal of Oceanography and Limnology*, Bellini’s work was visible to the international community. Therefore, a microbiology graduate student at the University of Massachusetts at Amherst, Richard Blakemore, published in *Science* the same observations about magnetosomes, changing the name in magnetotactics, but using an electron microscope. Blakemore was working in the Woods Hole Oceanographic Institution, in whose collections the pertinent publications of the Institute of Microbiology of the University of Pavia were extant, but he did not mention Bellini’s research in his own report. Unfortunately, these kind of accidents, like Rosalind Franklin’s one, are common in the scientific community, also in times of peer reviews, especially when the discovery is important. Nowadays, magnetosomes are the object of several applications, such as in chemotherapy, immunotherapy, and gene therapy, at pre-clinic stage also in patients. Their structures have been differently functionalized in accordance with the target, utilizing the presence of various chemical groups at their surface.

We were interested in the production of magnetic nanoparticles, consisting of suspensions containing chains of magnetosomes. A magnetosome is defined as an intracellular, membrane-bounded magnetic iron-bearing inorganic crystal. In particular, we considered chains of magnetic nanoparticles that were extracted from magnetotactic bacteria. These magnetotactic microorganisms are interesting and unique, since they possess flagella and other typical characters, but also contain structured particles, rich in iron, within intra-cytoplasmic membrane vesicles. These contents impart a magnetic response to bacterial cells. Furthermore, though bacterial magnetosomes are true prokaryotic organelles, they display a comparable degree of complexity to their eukaryotic counterpart. In fact, magnetosome

formation requires a complex process, involving vesicle formation, extracellular iron uptake by the cell, iron transport into the magnetosome vesicle, and biologically controlled magnetite or greigite mineralization within the magnetosome vesicle. Magnetic nanoparticles may be preferred for their unique characters, like high field irreversibility, high saturation field, superparamagnetism, extra anisotropy contributions, or shifted loops after field cooling. These phenomena arise from narrow and finite-size effects and surface effects that dominate the magnetic behavior of individual nanoparticles. When tested, magnetotactic bacteria evidenced larvicidal, pupicidal, and dengue viral effects of extracellular synthesized magneto-nanoparticles against *Ae. aegypti* mosquitoes. Magnetic nanoparticles proved to be highly toxic to chloroquine-resistant *Plasmodium falciparum*, dengue virus (DEN-2), and their mosquito vectors (Murugan et al., 2016d).

Following the research line the selection of the material necessary to obtain the nano-mosquitocites. Another important task in green nanotechnology is. In this way, several materials were tested. In particular, mangrove was elected to obtain green-mediated synthesis of silver nanoparticles with high HIV-1 reverse transcriptase inhibitory potential (Kumar et al., 2016a, b). In another paper, the mangrove *Sonneratia's* alba-synthesized silver nanoparticles reported the capacity to magnify guppy fish predation against *Aedes aegypti* young instars (Murugan et al., 2017b).

Among them, we proposed (Roni et al., 2016; Murugan et al., 2016a,b, c,e,f,g,h) a novel method of biofabrication of silver nanoparticles with insecticide activity against malaria vectors using eco extracted from crab shells. Again, it was decided to explore a multiple approach regarding the activity of the nanoparticles, including the impact on non-target organisms in an aquatic environment. First, the insecticide effects were experimented on against larvae and pupae of *Anopheles stephensi*, obtaining LC_{50} ranging from 3.18 ppm (I) to 6.54 ppm. Second, antibacterial properties of Ch-AgNP were proved against *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Salmonella typhi*, while no growth inhibition was reported in assays conducted on *Proteus vulgaris*. In terms of non-target effects, in standard laboratory conditions the predation efficiency of *Danio rerio* zebrafishes was tested, obtaining 68.8% and 61.6% against I and II instar larvae of *A. stephensi*, respectively. However, when chitosan-nanoparticles were added to the environment (Murugan et al., 2016b, Murugan et al., 2017a,b,c), fish predation was boosted to 89.5% and 77.3%, respectively. However, deleterious effects of aquatic environment on the non-target crab *Paratelphusa hydrodromous* were observed when quantitative analysis of antioxidant enzymes SOD,

CAT, and LPO from hepatopancreas of fresh water crabs exposed for 16 days to a nanoparticle-contaminated aquatic environment was conducted, highlighting some risks concerning the use of nanoparticles in aquatic environments at least for some organisms present in that environment (Murugan et al., 2016b). The biosynthesis, mosquitocidal, and antibacterial properties of *Toddalia asiatica*-synthesized silver nanoparticles were reported, and these did not impact predation of guppy *Poecilia reticulata* against the filariasis mosquito *Culex quinquefasciatus* (Murugan et al., 2015f).

A delicate argument concerns the effects of the release of nanoparticles on the habitat. Most concern is nowadays focused on the toxicity on predators of mosquito vectors, but other consequences cannot be excluded. In another paper, we reported utilization of seaweed in the synthesis of silver nanoparticles. The nanoparticles were thus obtained and their impact against the filariasis vector *Culex quinquefasciatus* was observed. Lymphatic filariasis is a parasitic infection that leads to a disease commonly known as elephantiasis, affecting nearly 1.4 billion people in 73 countries worldwide. Also in this case, negative effects on human health and the environment must be considered, since control against mosquito larvae is obtained by treatment with organophosphates and insect growth regulators. The frond extract of the seaweed *Caulerpa scalpelliformis* was utilized in the formation of nanoparticles (Murugan et al., 2015c). Once the toxicity of the seaweed extract and silver nanoparticles had been assessed against the filarial vector *C. quinquefasciatus*, the experiments were focused on the predatory efficiency of the cyclopoid crustacean *Mesocyclops longisetus* (Murugan et al., 2015e). This a copepod predator of mosquito larvae, as in the case of already reported. In a nanoparticle-contaminated environment, predation efficiency was 84% and 63%, respectively, on I and II instar larvae of *C. quinquefasciatus*, compared to 78% and 59% in the control treatment. The study showed that seaweed-synthesized silver nanoparticles use lead to little detrimental effects against aquatic predators, such as copepods (Govindarajan et al., 2015; Murugan et al., 2015a), including other *Mesocyclops* species (Chandramohan et al., 2016; Murugan et al., 2015c, 2015g).

An attempt to examine different effects on the habitat was made in a study of control of dengue by nanoparticles (Murugan et al., 2017a,b,c), in order to obtain the potential of mangrove extract (*Sonneratia alba*)-synthesized silver nanoparticles (AgNP), as eco-friendly nanoformulations effective against dengue virus and its mosquito vectors, as well as the effects on usual predators of the same habitat. AgNPs were obtained by a cheap method relying on *S. alba* extract as a reducing and stabilizing agent. First, the activity of the *S. alba* extract alone against *Aedes aegypti* was obtained,

with LC_{50} values that ranged from 192.03 ppm (larva I) to 353.36 ppm (pupa). However, the AgNP toxicity turned out to be much higher, since the LC_{50} ranged from 3.15 (I) to 13.61 ppm (pupa). Later, effects of predators of the vectors were tested with sublethal doses of AgNP, obtaining magnified predation rates of guppy fishes, *Poecilia reticulata*, against *Ae. aegypti* and *Chironomus kiiensis* larvae. Mangrove-fabricated AgNPs were also evaluated for their antimicrobial potential against *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Salmonella typhi*. *S. alba*-synthesized AgNPs tested at doses ranging from 5 to 15 $\mu\text{g}/\text{mL}$ downregulated the expression of the envelope (E) gene and protein in dengue virus (serotype DEN-2), while only small cytotoxicity rates (<15%) were detected on Vero cells when AgNPs were tested at 10 $\mu\text{g}/\text{mL}$ (Meenakshi and Jayaprakash, 2014).

After the tiger mosquito, it is time to meet the tiger frog. This study is based on a synergistic approach using biocontrol agents and botanical nano-insecticides for mosquito control. With single-step green synthesis, silver nanoparticles were obtained using the extract of *Artemisia vulgaris* (the same genus of the antimalarial *A. annua*) leaves as a reducing and stabilizing agent (Govindarajan and Benelli, 2016; Benelli et al., 2016). The mosquitocidal properties of *A. vulgaris* leaf extract inserted in green-synthesized nanoparticles against larvae and pupae of *Ae. aegypti* were investigated. The nanoparticles proved to be highly toxic to *Ae. aegypti* larval instars (I–IV) and pupae, with LC_{50} ranging from 4.4 (I) to 13.1 ppm (pupae). To evaluate the habitat disequilibrating effects, it was also necessary to investigate the ecologic effects of the green nanoparticles on the predatory efficiency of Asian bullfrog tadpoles, *Hoplobatrachus tigerinus*, against larvae of *Ae. aegypti*, under laboratory conditions and in an aquatic environment treated with ultra-low doses of the nanoparticles (Murugan et al., 2015b). *Ae. aegypti* and the bull frog actually share the same aquatic habitat, and the frog is fundamental in the biological control of this vector of dengue and chikungunya diseases. In the laboratory, the mean number of prey consumed per tadpole per day was 29.0 (I), 26.0 (II), 21.4 (III), and 16.7 (IV). After treatment with nanoparticles, the mean number of mosquito prey per tadpole per day increased to 34.2 (I), 32.4 (II), 27.4 (III), and 22.6 (IV). Despite the positive data, there are several aspects to be considered before any further steps. We do not know the reason for the increase in predatory activity and possible collateral effects. Any information on the behavior of any participant in the project must be considered on the basis of previous experiences. The Asian bullfrog (*Hoplobatrachus tigerinus*) is native to the Indian sub-continent and it is rapidly invading the Andaman archipelago, the Bay of Bengal, after its recent introduction. Rapid predation by larval *H. tigerinus* resulted in no survival of

endemic frog tadpoles previously present in that habitat. The tadpoles of the tiger frog are voraciously carnivorous, attacking any prey, including two species of endemic anuran tadpoles, *Microhyla chakrapanii* and *Kaloula ghoshi*, in a sort of cannibalism. Survival of *H. tigerinus* was density-dependent. Therefore, invasive populations, or potential induced incursions of alien species for biological control, can generate important ecological variations. Already Darwin had observed many years previously the potential impact caused by the loss of even a single species.

In the case of mosquito control, these activities must also consider recent important challenges, such as the recent outbreaks of novel arbovirus, including the development of resistance in several Culicidae species and the spreading of alien invasive mosquitoes in several regions. In particular, microbiopesticides must be joined with other measures, such as personal defenses by insecticide-treated bed nets and repellents made from natural products. Further strategies may consider bio-agents active against mosquito young instars (i.e., selected species among fishes, amphibians, and copepods), sterile insect techniques (SIT) including boosted SIT, symbiotic-based methods, and transgenic-produced mosquitos. These measures must be integrated, since no single method has produced fully successful or satisfying results.

The neem nano-emulsion can be easily obtained thanks to the oily nature of seed extract and used as an antimicrobial agent. However, the structure of the active components, such as the limonoids of neem (see [Chapter 7](#)), are subjected to rapid attack by oxygen and humidity when exposed to air, and their permanence is important to obtain a good insecticide effect. Green-fabricated nanoparticles have been studied as promising toxic agents against mosquito young instars, and as adult oviposition deterrents in aquatic environment merits. The idea was to treat the water with ultra-low doses (e.g., 1–3 ppm) of green-synthesized nanomosquitocides, which reduced the motility of mosquito larvae. Nanoparticles are important to avoid degradation of limonoids in the open air, since azadirachtines are reported to have a half-life of a few days, giving rise to the need for costly several treatments. Biodegradability is mainly due to exposure to the sun and to humidity. This is an advantage, avoiding the problems like DDT due to the accumulation in the habitat, but it is one of the more important limits in neem utilization. Therefore, nanotechnology can be used to protect active constituents and also to facilitate interaction with the target organism.

At the end of this short review of selected examples of utilization of nanoparticles in control of insect-borne disease vectors, it is necessary to underline some points of interest. Nanotechnology is still in its infancy,

and its real perspectives are still in progress. In particular, the quantity of positive results obtained using organic matter of different origins can be suspicious. However, parasitism is relevant to all kinds of organisms, in particular plants that must depend on their chemical arsenal. Therefore, the real task is the exact chemical determination of the active constituents, often largely lacking, and the mechanisms of action necessary for the certification of their utilization. Only through these advancements will the reality of nanotechnology in insect-borne diseases achieve completely its fundamental role and importance, moving on from the initial step of the pure experimental phase. Meanwhile, it is necessary to join the market and obtain applications of green nanotechnology products on a large scale, once their safe and eco-friendly effects have been confirmed and certified.



Integrated protection programs

As previously reported, in-depth knowledge of the habitat network is one possible tool to develop an efficient and reliable pest control system. As evident in the *Xylella* episode (see [Chapter 5](#)), all the components must be considered interconnected and treated in the logic of the superorganism: (a) the soil, subjected to radical changes in its composition by the climate impact or human abandon or intervention; (b) the host, whose natural defenses are the first and best bulwark against infection and disease development; (c) the etiological agent, considered not only as the responsible of the disease but as a voluble environmental agent whose actions are moved by homeostatic reasons, which can be also considered as part of an useful control strategy; and (d) the vector, the ideal target being visible and accessible, but also acting as a delicate part of the network. In this scenario, the reason for the adopted measures cannot be reduced to the wellness of the host, and information about the tendency of the phenomenon must be obtained, including the main factors governing the changes. It is complicated, but it is the only way to avoid disasters. The programme must consider the key importance of the vectors. Monitor the harmful organisms, selecting different methods and appropriate times, as well as the appropriate chemical groups, taking into account the growth period and the presence of natural enemies. Selective insecticides should be developed and areas where harmful insects susceptible to insecticides can reproduce should be provided. The expected result is that the harmful organisms should mate those resistant and in this way decrease the portion of the resilient genes inside the population.

Regarding integrated control programs in agriculture, many different conventional and sustainable strategies can be incorporated, including application of insecticides correctly and at the right time, pest monitoring, resistance management, protection of beneficial insects, cultural practice, crop rotation, pest-resistant varieties (even transgenic when allowed), and chemical attractants or deterrents derived where possible by natural products to avoid an insurgence of resistance.

Great attention about novel methods of control avoiding synthetic insecticides is focused on targeting the causative vectors and pathogens by biological control. The aim is avoid the consequences of massive utilization, as environmental damage, destruction of useful insects, and resistance decided the decline of such substances to combat mosquitoes that were once considered the most effective way of controlling mosquito-borne diseases. The current, simplest, and generally accepted definition for biological control is an environmentally sound and effective means of reducing or mitigating vectors and vector effects through the use of natural enemies. The different biological control agents being studied in different parts of the world for the control of vector mosquitoes include many naturally occurring predators, parasites, and pathogens of vector insects, including fungi and bacteria. However, although biological control should provide an effective and eco-friendly approach, which can be used as an alternative to minimize the mosquito population and can provide an effective and eco-friendly approach to mosquito control, the results in many cases were not satisfactory. The selection of natural enemies can include also alien species, the autochthonous ones not being sufficient. However, the effects of such introductions must be carefully considered.

Phylum	Arthropoda
Class	Insecta
Order	Hemiptera
Family	Pentatomidae
Genus	<i>Halyomorpha</i>
Species	<i>H. halys</i>

If you have occasion to travel in Italy in the central region, Emilia-Romagna, you may well notice the presence of large cultivations of fruit trees, mainly apple and pear trees. It is a flat land, traversed by the river Po, the largest in Italy, where important and historic towns such as Bologna, Ferrara, and Padova alternate with cultivated fields. In these territories, the

apple trees, which are nowadays more profitable for the market, have substituted hemp, tobacco, and mays, generating a monoculture, or better a monovariety cultivation, with very few utilized cultivars among the cultured apple trees. Simplified habitats are more vulnerable. Furthermore, this situation is nonsense, considering the hundreds of varieties characterizing *Pyrus malus*, as the result of careful and patient work of farmers and the plastic genome of this species. In the Arboretum of Alma Ata in Kazakhstan, considered the homeland of the apple, the precious genomes of hundreds of cultivars are still served, but the world market is focused on a few types, such as Golden Delicious and Fuji.

This part of Italy was always rich and productive, in terms of ideas and art as well as delicious food, but in the last 8 years the situation has changed under the real threat of an alien uninvited guest. The brown marmorated stink bug (*Halyomorpha halys*) is menacing 60%–70% of fruit production, gorging on unripe fruit. The bugs have sunk their needle-sharp stylets into the pear, apple, and peach fruits, creating wounds evident on the surface, producing a clear, sugary goo. They form corky brown blemishes and leave the trees more vulnerable to infection. This insect is native to Orient Asia, but since 1998 has been accidentally introduced first in many parts of North America, and has recently become established in Africa, Europe, and South America. One hypothesis is a transportation of eggs from China or Japan occurred as a stowaway in packing crates or on various types of machinery, again from China or Japan. The nymphs and adults, feeding on more than 100 species of plants, have seriously affected many agricultural crops in the Mid-Atlantic United States, where in 2010, \$37 million dollars in apple crops were lost, and some stone fruit growers lost more than 90% of their crops, including, in addition to fruit trees, corn, soybeans, berries, and tomatoes in 43 states of the USA. The brown marmorated stink bug is a sucking insect, belonging to Hemiptera, the “true bugs.” Through its proboscis, it pierces the host plant to feed, causing leaf stippling, seed loss, and possible transmission of plant pathogens causing several devastating diseases. The formation of dimpled necrotic areas on the outer surface of fruits causes the main damage in terms of market appeal, with consumers looking for perfectly shaped fruits in their shops.

The diffusion of the brown marmorated stink bug, like many invasive species, is benefited by the absence of major enemies in its new home to keep its population in check. The description “marmorated” is derived from the gray color of the insect, although there are common species colored in green. These insects are known for their tendency to escape the cold season

by finding shelter inside farms and houses, where they are found when they are flying and for their pungent odor when disturbed or crushed or simply when one attempts to move them. This shield-shaped stink bug alien invasive pest is different from the indigenous species due to its indiscriminate appetite and its coriander-like odor, due to two simple aldehydes: trans-2-decenal and trans-2-octenal. The stink bug emits its smell through holes in its abdomen, as a defense to prevent it from being eaten by birds and lizards. However, simply handling the bug, injuring it, can trigger it to release the odor. After several conventional attempts to stop this pest, all hopes now focus on biological control, consisting in the release of the stink bug's natural enemy. The choice is the samurai wasp (*Trissolcus japonicus*), which despite its very small shape is very effective, being a pitiless and systematic killer. The stink bug's nemesis, the wasp injects its own eggs into the stink bug's, leaving larvae that eat the developing bugs before chewing their way out. Currently, the samurai strains of these wasps are imported from Asia, but in 2005 the taxonomist Elijah Talamas, at the Florida Department of Agriculture and Consumer Services in Gainesville, identified native wasps parasitizing stink bug eggs in Maryland, USA. The most convincing hypothesis is that bug and wasp both migrated on their own, and in future they will participate their struggle for life. However, this is not automatic, since new habitat conditions are different from the starting ones. Therefore, many nations have begun to regulate strictly the release of biocontrol agents, which can include insects, fungi, and bacteria, and require studies to predict potential "nontarget effects." Meanwhile, the only real measure is severe and strict controls on imports of goods and organisms. Thus, a whole cargo of Ferrari cars (the house of Ferrari is at Modena in Emilia-Romagna) was prevented from entering in Australia because of three exemplars of marmorated stink bug found in the cargo.

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