Joseph Rossano

BOLD: Costa Rica

CONTENTS

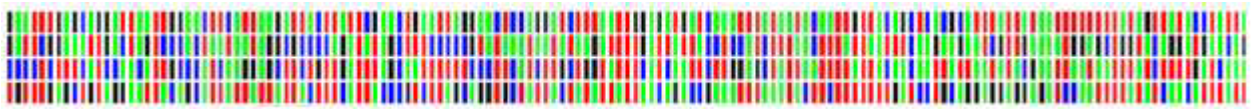
About the project:

The Ontario Genomics Institute has partnered with renowned Seattle-based artist, Joseph Rossano, and biologists Dr. Paul Hebert and Dr. Daniel Janzen to engage the public around the science of DNA barcoding and how it is being used to catalog the world's vast – and threatened – biodiversity.

Among the many applications of DNA barcoding is its use as an important tool in modern conservation biology. Indeed, conservation is at the very core of this work, which provides viewers with the opportunity to reflect on the impact of humankind on our environment.

What is a DNA barcode?

If you look closely at the side of the encasement on these works of art, you'll see a series of A's, C's, G's and T's. They make up a DNA sequence, but not just any sequence – it's a sequence unique to this species. Each species has a different sequence at this particular spot in their DNA code. Scientists call this sequence fragment a "DNA barcode". If each part of the sequence were represented by a different colour, it might look like:



DNA barcoding uses a small fragment of a single gene in an organism's DNA to identify the species to which that organism belongs, much like one might use a UPC barcode to distinguish different products. These powerful tools are helping scientists to catalogue the world's biodiversity. The process began in Guelph, Ontario, Canada, and scientists here continue to lead international work aiming to catalogue the earth's life forms completely.

About the Area de Conservación Guanacaste (ACG)

A UNESCO World Heritage Site since 1999, the Area de Conservación Guanacaste (ACG) in Costa Rica is a vast protected ecosystem with an area of 120,000 terrestrial and 70,000 marine hectares. The ACG contains important natural habitats for the conservation of biological diversity – approximately 230,000 species in total – including the best dry forest habitats from Central America to northern Mexico and key habitats for endangered or rare plant and animal species. The site demonstrates significant ecological processes in both its terrestrial and marine-coastal environments. (**modified from UNESCO*)

The mission of the ACG is to conserve the biodiversity of the ecosystems and the cultural heritage present in the ACG, as a model of development which integrates society in the management of the Area.

About the sculptures: A note from the artist, Joseph Rossano

As an artist, I strive to distil ideas, concepts, and reality into their bare essence. My resulting minimalist sculptures, I hope, convey an emotion, ask a question, or direct the viewer on a path of introspection and investigation, as they explore man's impact on the environment.

My series "BOLD" is named for the acronym for the Barcode of Life Data Systems (BOLD) database. The subject of each specimen box is neither real nor is it an accurate representation of the creature it is designed to represent. The subjects of these sculptures are a jewelled representation of reality that draw the viewer in for a closer inspection. As the viewer shortens the distance between himself and the sculpture, the specimen becomes increasingly difficult to discern. The viewer, now confronted with the frustration of being unable to make out exactly what is in the box, discovers the clear and legible text surrounding the specimen.

What is the text on the side of each piece? The text is the 100% accurate representation of the specimen that attracted you. The text is the FASTA file, a textual representation of the DNA barcode that identifies the unique species.

What is the story of this specimen? What is a DNA barcode? I'm not a scientist; my role in this interactive collaboration is to distil reality into a visual hook that leads you to the answers.

Welcome to BOLD.

BOLD-1

About *Zerene cesonia* – by Dr. Dan Janzen

I grew up in the outskirts of Minneapolis (before there were suburbs) in the early 1950's. I still remember my first caterpillar – a green thing feeding on the leaves of a shrubby legume in a glade in a city park (quite different from the aposematic monarch butterfly caterpillar reared by so many children). Out of it came a fine yellow so-called "dog's head" butterfly – *Zerene cesonia*. Minneapolis is a long way from Costa Rica – ecologically, geographically, or even as the crow flies. What we know is that *Z. cesonia* is not migrating back and forth as do monarchs, but rather that it is a steadily present denizen of the southern US, and its population creeps northward each summer, only to be blasted back by the blizzards each winter (see the gorgeous range map here; *Z. cesonia* is a breeding population scattered across the entire intervening landscape between Minnesota and Costa Rica, but on the dry side, where shrubby legumes are commonplace. It may follow pastures into the Area de Conservación Guanacaste (ACG) rain forest on occasion, but it needed humans to make the rain forest ecosystem habitable for it).

The males of *Z. cesonia* all seem to be about one color and the one pattern that we figure, but as is commonplace among species of *Pieridae*, the females range from pale to dark yellow, and have a highly variable light to dark black pattern. Don't ask for speculation about the biological significance of the seeming eye spot on the top and under of the forewing – I suspect that it is not playing off the same predator avoidance as the false eye spots on forest caterpillars and pupae, and even the wings of many species of ACG *Nymphalidae*. The spot does have, however, much in common with the same kind of spot on the wings of many other medium-sized pierids (other than the extreme mimic such as *Dismorphia amphione*).

BOLD-2

About *Belemnia trotschi* – by Dr. Dan Janzen

This day flying moth is in the "unbelievably gaudy" category. All that color is no accident of pigment physiology. *Belemnia trotschi* is undoubtedly saying something to the world, but what? The widespread belief among entomologists is that such bright colors are aposematic – colors that warn the observing monkey or bird not to touch or eat. This may well be the case, but there are caveats. First, the known Area de Conservación Guanacaste (ACG) food plant of the very cryptic caterpillar is *Brosimum guianense* (*Moraceae*). The fruits, seeds and foliage of this large tree are quite edible to vertebrates. The inference is that if this arctiid moth is toxic to the touch, taste or gut (as are believed to be many arctiid moths), it is very likely to be manufacturing its own toxins rather than merely extracting or sequestering them from the caterpillar food plant.

Second, we need to ask if the colors are displayed ostentatiously, or does this moth in nature actually match some gaudy background, like a flower or ripe fruit? The short answer is that the the adults do walk and fly ostentatiously in the full sun over foliage and flowers of many colors, but none of them are color matches for the moth. The moth is unambiguously displaying a flag. Third, a gaudy arctiid may be a Batesian mimic, quite edible but looking much like another truly obnoxious species. In the case of *B. trotschi*, it is unique, not looking like anything else in any tropical habitat known to us – though one can never fully discard the possibility that at the time of evolution of these colors, or in some other part of its range, there were a host of other look-alikes – Batesian and/or Mullerian mimics. Fourth, it may be that rather than these colors being an exact match of anything, they are simply a gaudy mess of bright signal that says to the predator's genes or learning experiences "I am one of those bright gaudy things that you don't want to mess with". As such, it would be sharing a defense strategy with many tens of species of diurnal *Arctiidae, Riodinidae, Noctuidae, Geometridae*, and *Nymphalidae*.

BOLD-3

About *Tithorea pinthias* – by Dr. Dan Janzen

At first glance, *Tithorea pinthias* (also known as *Tithorea tarricina pinthias*) is just one more toxic-to-eat Mullerian mimic flapping or sailing along a rain forest trail, with its rusty red hind wings and black-with-yellow-spotted forewings. And comparing it, even in detail rather than the blur of flight, with *Heliconius hecale* just reinforces that conclusion. And as has been said before and will be said again, in addition to the classical concept of mimicry as largely a learning-based phenomenon, it may well be that the birds are even genetically hard-wired to ignore this color pattern.

But the story is broader. In 2005, an entire issue of *Nearctic Lepidoptera* was devoted to the regal fritillary, *Speyeria idalia*. This very large and very attractive endangered US species of nymphalid butterfly once ranged widely in the northern half of the US east of the Rocky Mountains. It was just about everywhere that there was tall grass prairie accompanied by the violet leaves (*Violaceae*) that its caterpillars eat. Now it is reduced to a few enclaves dotted over that once large distribution. *S. idalia* is an outstanding color pattern, not duplicated elsewhere in Kansas, Indiana or the Dakotas. Mimicry never crosses your mind. Yet there is a mental itch. The butterfly has relatively slow, flapping and ostentatious flight. It perches on flowers with its wings wide open, advertising to the world. And its flight is a blur of orange, and black with white/yellowish/silver spots. Its hind wings are black with white spots, and the forewings are orange (with some scattered black etchings). In short, it is *Tithorea pinthias* or *Heliconius hecale* inverted. But you feel that the predators of *T. pinthias* are in the neotropics, not Kansas? When the birds migrate north in the spring, they do not leave behind their genetics and their knowledge.

BOLD-4

About *Azeta rhodogaster* – by Dr. Dan Janzen

If you see a noctuid moth in Area de Conservación Guanacaste (ACG) with a red abdomen, it is *Azeta rhodogaster*. There is no other like it. Equally, as the bright red abdomen suggests, this species is rather more diurnal and rather more inclined to fly when a predator approaches, than are many hundreds of other species of ACG Noctuidae. It perches exposed on the foliage, watches for movement with its large eyes, and flees when approached. It is very likely that the red abdomen functions as do the yellow hind wings on so many noctuids: The searching bird zeroes in on the red as the moth is in flight, but the red abruptly disappears when the moth alights.

And the caterpillar of *A. rhodogaster* also steers the researcher away from aposematism. The pale greenish-yellowish-with-black dots caterpillars are host-specific to *Gliricidia sepium* (shown), a small to very large native tree of ACG dry forest. This dry forest tree has been introduced around the global tropics, in both dry and rain forest, as a living fence post. When this tree was planted as the support for barbed wire fences in the rain forest side of Costa Rica, *A. rhodogaster* was just one of the many dry forest species that moved from dry to rain forest pastures, following its food plant.

A. rhodogaster caterpillars can reach quite spectacular abundances, defoliating their food plants if they are just saplings or young treelets. At these times, it is easy to collect many of the caterpillars so as to see what parasitoids are using them and it is apparently also easy for their parasitoids to find them. In one sample of 446 mostly last instar caterpillars collected during a population explosion from a few *G. sepium* treelets in ACG dry forest, *Eucelatoria armigera*, a medium-sized somewhat generalist tachinid fly, killed at least 40 percent of the caterpillars. Interestingly, this species of caterpillar does not seem to have any species of parasitoid that is a specialist on it.

BOLD-5

About *Adelpha basiloides* – by Dr. Dan Janzen

If there was ever a mimetic mess among the adult Area de Conservación Guanacaste (ACG) Nymphalidae, it is *Adelpha*. Some 16 of the 19 ACG species reared to date look like one or more of each other (especially when flying), except for the two cloud forest species, *Adelpha tracta* and *Adelpha demialba*. These two deviate from the lowland generic morph, as is commonplace when there is a group with many lowland species and a few occupying upper elevations. And the "*Adelpha* color pattern" – here portrayed by *Adelpha basiloides* - plays out in seemingly infinite minor variation over more than 100 other Neotropical species (with even a few in North America). But among all of these in the ACG, there was one that was common, distinctive for a single trait in the forewing - *Adelpha basiloides*. The distinctive split white cell at the top of the column of white cells marching up the forewing separates it from all the others.

That is, until we got its DNA barcodes. What the world knows as *A. basiloides* is clearly two entities in ACG. One, now known as *Adelpha* basiloidesDHJ01 until we can figure out what it really is, is basically the dry forest species, while *Adelpha* basiloidesDHJ02 appears to be primarily occupying the interface between rain forest and dry forest. But the two certainly can be found in the same place and the caterpillars feed on the same species of plants.

But what does that orange and white pattern on a dark brown background mean in the life of an *Adelpha*? It is easy to suspect that it is indeed an aposematic coloration, both because these butterflies fly, float, flap and perch in the vicinity of fly-catching birds that certainly should have the ability to catch *Adelpha*, yet are ignored by the birds. But this is yet one more case where only detailed, and perhaps somewhat unpleasant, experiments could determine if they are models, mimics or both. It will not be fun to feed them to birds.

BOLD-6

About *Siderone galanthis* - by Dr. Dan Janzen

Staring idly into the top of a dry forest oak tree on a long hot dry season afternoon, one comes to the sudden realization that there is a brilliant red flapping dot that appears periodically against the blue sky. For an hour it fools you into thinking that it is an *Agrias amydon* repteatedly launching and perching on the sap flow from a wounded *Quercus oleoides* branch – which is festooned with what appear to be flaps of bark but are actually slightly tipsy *Memphis*, *Anaea*, *Archaeoprepona*, *Prepona*, *Historis* and *Myscelis*, all congregated there to suck up the yeast-ridden fermenting sap (a.k.a. beer), and in the process transmit that same yeast to other newly damaged tree branches. But for some unknown reason, perhaps because it is shoved to the side by more aggressive butterflies, the red winged butterfly keeps leaping into the air for several turns before re-alighting. And then a pair of binoculars is produced and the red thing is pronounced to be *Siderone galanthis*. The underside, like that of the other sap feeders, looks for all the world like a dead leaf or a dead flap of tree bark but the top bears the brilliant eye-catching red (as in blue for others, or even red and blue for *Agrias amydon*) that says, once the bearer is in the air, "don't bother to try, yes, here I am, but I am way too fast". However, again as with all these other species that catch the human eye so visibly, we really do not know to what degree the evolution of these colors has been intertwined with the evolution of courtship displays.

BOLD-7

About *Othorene* veranaDHJ01 - by Dr. Dan Janzen

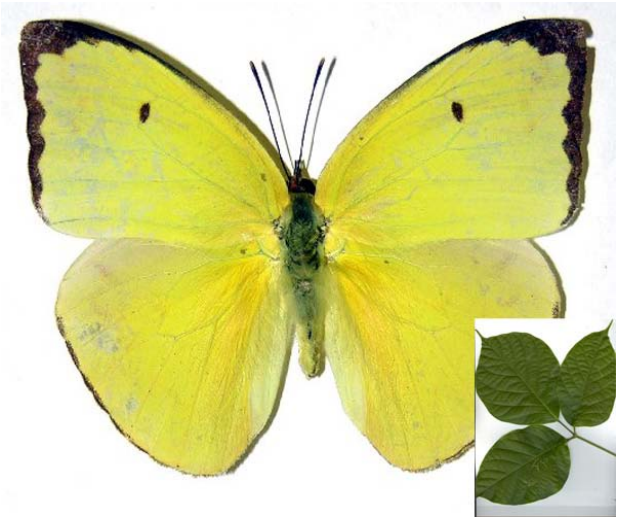
In the early 1980's, when the Area de Conservación Guanacaste (ACG) caterpillar inventory was newborn and focused on the dry forest of Sector Santa Rosa, the large caterpillars of Saturniidae fell quickly to the searchers. Simultaneously, the adults at the light traps quickly tallied up to an asymptote – more nights and more years did not generate more species for the dry forest saturniid list. There were 29 species at the lights and 28 species of caterpillars. Where was the missing caterpillar of *Othorene* veranaDHJ01? Common at the lights, though often confused by collectors with its look-alike *Othorene purpurascens*, *O.* veranaDHJ01 "must" have a common and large caterpillar, but it simply was not being found by all an ever increasing number of people.

So, in 1984, in frustration, the inventory broke its own rule of not explicitly searching for any particular species (such explicit search is good way to seriously lower the inventory yield per dollar of US tax dollars spent). Several plump egg-filled and fertilized females were caught at the lights, and they laid – as saturniids are wont to do – hundreds of eggs glued to the insides of their plastic bag cages. Six days later they hatched and five of the hungry first instars were put into a small plastic bag with several leaves of a species of tree – 300 species to be exact.

The caterpillars rejected all species of tree leaves except those of *Quercus oleoides* (shown), the single common lowland tropical Central American oak (actually, *Q. oleoides* is really the same species as *Q. virginiana*, the Virginia live oak of the eastern and southern US, which magically changes its name to *Q. oleioides* about where it crosses the border into Mexico). This evergreen oak once covered tens of thousands of hectares of ACG dry forest and still maintains what appears to be a healthy breeding population, which we now know supports a healthy breeding population of *O.* veranaDHJ01.

BOLD-8

About *Aphrissa statira* - by Dr. Dan Janzen



"The butterflies are merciless today" said a cartoon burned into memory but lost to the bibliographer. And so they were in late June, looking west down the channel of the paved entrance road at the official entrance to Area de Conservación Guanacaste (ACG) at the Casetilla Entrada. It was a river of large bright yellow butterflies pouring out of Sector Santa Rosa's dry forest at the end of their first generation of the year. They passed in ones to 20s every few seconds, flying 1-4 m above the ground, males and females, the children output of parents that had arrived at the ACG dry forest a month earlier to lay their eggs on the new foliage of the beginning of the rainy season. *Aphrissa statira* was one of the more common members of this mass of large Pieridae, along with five species of *Phoebis* and several more genera.

When ACG was one unbroken stretch of old-growth forest, from the Pacific coast to the Caribbean lowlands, the entrance of large yellow pierids with the first rains, their single dry forest generation, and their massive exit to wetter areas to the east, was probably very cleanly performed. Today, a few individuals of some species stay behind, though not *A. statira*, to have 1-3 minimalist rainy season generations on the small ocean of food plants in the early successional edges of dry forest roadsides and pastures. *A. statira* is a specialist on the very new foliage of the old-growth huge and long-lived forest vine *Callichlamys latifolia* (shown). The foliage-green caterpillars are both very abundant and very evident to any foraging bird, both because they perch on both sides of a leaf, and because their damage is often the first to be sustained by the new large leaves of their food plants. However, there is safety in numbers and the forest at this time is rich in green edible caterpillars, apparently often satiating their avian predators.

BOLD-9

About *Bungalotis diophorus* - by Dr. Dan Janzen

We have never seen an adult *Bungalotis diophorus* skipper free-flying in Area de Conservación Guanacaste (ACG). But, they surely are there – their biological tracks, otherwise known as eggs, larvae and pupae – are quite common in the ACG rainforest.

Why do we not see the very different-appearing male – which strongly resembles the male of *Bungalotis astylos* – and female *B. diophorus* adults? We suspect that they fly in the very late afternoon when it is nearly dark, or even at night, though this is less likely because none have been collected at lights, at least to our knowledge. The female, with her bright white spots, is probably very visible to the monochromatically red-brown male even in the low light of evening. If the wing is viewed at a steep angle, many, but not all, of the white spots virtually glow white – like a bicycle rear reflector or a joggers white reflective protective shoes.

Here we identify this butterfly as *B. diophorus*, but we suspect that it really is an undescribed species. The problem is that it looks very much like *B. diophorus* from Brazil, a specimen of which is the holotype for the species name. It looks so much like it that almost anyone would identify the ACG species as that. However, the project skipper taxonomist – John M. Burns at the Smithsonian Institution – has found slight differences between the ACG specimens and the Brazilian specimens, so we await a new name for it in the coming years. This example underlines a chronic problem with Central American butterflies – many closely resemble the type specimen collected in South America, and only very close taxonomic scrutiny, and often, substantial further collecting, can determine if there are one or two species under one name.

BOLD-10

About *Memphis mora* - by Dr. Dan Janzen

Memphis mora is a classical black-with-some-blue-iridescence charaxine, normally encountered by the butterflyologist when it has come to feed on fermenting bananas in a rain forest butterfly trap. The males of *Memphis* tend to do the black and blue one way, and the females do the black and blue yet another way, with generally more blue than have the males.

But *M. mora* holds a special place in Area de Conservación Guanacaste (ACG) Memphis natural history. The ACG rain forest is densely populated with many tens of species of Lauraceae – *Ocotea, Nectandra, Licaria, Beilschmedia,* and *Persea*. And many of these are fed on by the caterpillars of various species of *Memphis* (and other charaxines as well). But *M. mora* feeds on only one of them – *Ocotea cernua*. It is one among many and to a human eye and taste has nothing special about it. But clearly it does to the other Lauraceae-feeding species of *Memphis*, because of 110 *Memphis* caterpillars found to date by the inventory on *O. cernea*, every single one has been *M. mora*, and as mentioned above, a *M. mora* caterpillar has never been found on any other species of food plant (despite the inventory having found more than 1000 wild *Memphis* caterpillars).

BOLD-11

About *Morpho amathonte* - by Dr. Dan Janzen

A book about Costa Rican Lepidoptera would be incomplete without at least one image of a bright iridescent blue morpho butterfly. The usual one in tourist brochures is *Morpho peleides*, easily reared by butterfly farms and having a strong wide black band around the margins of the wings. *M. peleides* is also the species so commonly encountered flying at people-height down forest trails and along roadsides in the rain forest, dry forest and cloud forest. *Morpho amathonte*, however, is exclusively Area de Conservación Guanacaste (ACG) rain forest, neither venturing up the volcano into cloud forest nor over into dry forest, even though at least some of its caterpillar food plants – Fabaceae, Dichapetalaceae and even the occasional palm – are found in these other two "more extreme" ecosystems. *M. amathonte* males are quite distinctive in having no black border (except for the wing tip and forewing costa) around the intense field of blue, while the females do have a wide brown-black and spotted border to the blue field. Both sexes, with their larger wings than those of *M. peleides*, have a swooping flopping flight and often go as high as 10-30 m above the ground as they course up and down road cuts through the forest.

All of the ACG morphos – *M. amathonte, M. peleides, M. granadensis, M. polyphemus, M. theseus* – are blessed with a constellation of false eye spots on their undersides. The protective function of these eye spots is clearly not when in flight, but most likely when roosting on foliage, or when perched on rotting fallen fruit on the forest floor. They, like many saturniines and brasolines with similar false eye spots, offer a super stimulus on close view that says "you are being looked at, close up" – a stimulus that if not responded to by instant flight, will make lunch of the viewer.

BOLD-12

About *Mimoides clusoculis* - by Dr. Dan Janzen

Why the name *Mimoides*? It is hard to walk for an hour along a rainy season ACG trail in insolated secondary succession without encountering a large black butterfly with white patches on the forewings and red patches on the hind wings. It has a sufficiently fast wing beat such that the details of the white and the red are blurry, but the colors are obvious. It pauses here and there on foliage, tasting for the chemicals that suggest a potential site to lay an egg. It visits butterfly flowers, with the blue flowers of *Stachytarpheta frantzi* (Verbenaceae) very high on the list of favorites. But who are you actually looking at? There are at least 15 species of ACG mimetic Papilionidae with this behavior and color pattern (e.g., compare with *Parides iphidamus*).

Many of these mimics are so similar that even the very best butterflyologists have to catch the specimen to know for certain which species it is. Not all 15 visit the same flowers at the same time in the same ACG ecosystem, but since the birds circulate, this may not matter much. And intermingled with them are look-alikes from other families, such as day-flying pericopid moths (e.g., *Dysschema jansonis*, Actiidae), pierids (e.g., *Archoneas tereas*) and a host of nymphalids with red to orange on the hind wings and white to yellow on the forewings. The nymphalids do not match the textbook examples of quite exact mimetic resemblances of the papilionids, pericopids and pierids – and are generally ascribed to other mimetic complexes – but when in flight in bad light and to a not very mentally or visually discriminating bird, they certainly can easily be ascribed to the margins of the *Mimoides* mimetic color regime. And who is mimicking who? The classical center of this Mullerian and Batesian mimicry complex is *Parides*. However, to know if there even is a center will require a lot more field experimentation with real predators in the wild.

BOLD-14

About *Erbessa salvini* - by Dr. Dan Janzen

While the adult of *E. salvini* has gone the direction of one kind of aposematism or mimcry, the caterpillar has gone another. First, with its orange head and rear end, and green-pale striped-variegated body, it is a classical dioptine caterpillar, part of the huge mimicry ring of red-dark-white-red ACG caterpillars. Whether these are Batesian, Mullerian, or simply avoided, is yet to be discovered. But it has gone a step further in the never-ending evolutionary arms escalation. So many notodontid caterpillars have the terminal prolegs evolutionarily projected into other structures that it is almost a signature of the family. The most posterior prolegs of *E. salvini* are enormously extended into what can best be described as a white-tipped long black antenna, making the rear of the caterpillar appear to be the head end of some large insect.

But what is the value of having the rear look like the head? The classical answer is that if the predator strikes the more rubbery and less nerve-receptor-laden rear, the caterpillar has a higher chance of wiggling or falling away into the vegetation tangle below (and into which many a bird would be reluctant to dive in pursuit). However, the white tip may signal something more significant. The caterpillar of *Hylesia continua*, a common, highly urticating and often ostentatious black and white caterpillar in the same habitat, has the anterior black and white-tipped scoli enormously extended and clearly waves them about as part of its aposematic display. The rear of *E. salvini* may be evolutionarily locked into the same defense.

About the collaborators

Paul Hebert, PhD, a globally recognized pioneer of DNA Barcoding, is Canada Research Chair of Molecular Biodiversity and Director of the Canadian Centre for DNA Barcoding at the Biodiversity Institute, University of Guelph, Ontario, Canada. He is also Principal Investigator on the International Barcode of Life (iBOL) project.

Dan Janzen, PhD, is an evolutionary ecologist, naturalist, and conservationist, and Dimaura Professor of Conservation Biology at the University of Pennsylvania. For 56 years he has spent much of his time doing field research in Costa Rica and since 1985 has been a founder and technical advisor to Area de Conservación Guanacaste (ACG). ACG, 2% of Costa Rica and the size of New York City and all its suburbs, is the oldest, largest and most successful tropical habitat restoration project in the world, located just south of the Costa Rica-Nicaragua border.

Ontario Genomics Institute (OGI) is a private, not-for-profit corporation based in Toronto, Ontario, Canada, focused on using world-class research to create strategic genomics resources and accelerate Ontario's development of a globally-competitive life sciences sector. Through its relationship with Genome Canada, the Ontario Ministry of Research and Innovation, and other private and public sector partners, OGI works to: identify, attract and support investment in Ontario-led genomics research; catalyze access to and the impact of genomics resources; and, raise the visibility of genomics as well as its impact and associated issues.

To learn more please visit: http://www.ontariogenomics.ca/outreach/BOLD