When hybrids are fertile

Unlikely cross-breeds can create new species of plants and animals

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In addition to being talented, Darwin was lucky. Upon arriving in the Galapagos Islands in the Pacific Ocean, he found a rich variety of tortoises and birds living under unique environmental conditions, such as geographic isolation and diet, which must have strongly influenced their evolution over millions of years. The probable cause of the occurrence of so many animals that were so similar to each other – birds, for example, with shorter or longer bills, depending on what they eat – seemed clear. However, the entire world is not precisely similar to the Galapagos. Even if they study areas rich in biodiversity, such as the Atlantic Forest, biologists today do not always encounter evolutionary histories and closely related species that display such marked differences. Nevertheless, when working with specific strands of DNA sequences known as molecular markers, biologists can now identify the genetic bases of species diversification. A mechanism for the formation of new species that has been gaining recognition among researchers is the possibility of plant and animal species that are genetically similar to one other crossing naturally and generating fertile hybrids.

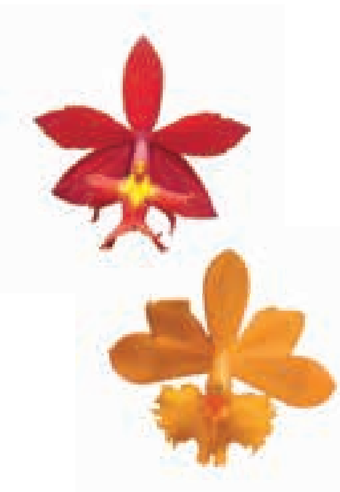
Previously, this idea was not widely accepted, because, in general, different species have different numbers of chromosomes, the structures within cells that contain genes. This difference can prevent the normal development of the embryo, as each chromosome that came from the male parent must be aligned with an equivalent chromosome from the female parent when the fertilized cell divides for the first time. Without this proper alignment, the cell usually cannot divide and, thus, dies. However, there are exceptions, and such exceptions appear to be less rare than was previously thought. The crossing between plants – or animals – of closely related species can produce offspring that, although of hybrid origin, are fertile, even though some chromosomes do not encounter their respective partner in the initial phases of cell division. With sufficient time and favorable environmental conditions, such hybridization events can generate species that are different from both of those from which they originated.

Today, the word "hybrid" defines the sterile mule, which is the result of a donkey crossing with a horse, but it also defines fertile organisms, such as the hybrid orchids from the Atlantic Forest that are maintained in a plant nursery at the Botanical Institute of São Paulo. This hybrid orchid has 38 chromosomes and resulted from a natural cross between two wild species, *Epi-*

EDUARDO CESAR

A hybrid orchid
from the Atlantic
Forest

EDUARDO CESAR



A hybrid with a flower in two colors: red like *Epidendrum puniceolutem* and yellow like *E. fulgens*

dendrum fulgens, which has 24 chromosomes, and Epidendrum puniceolutem, which contains 52 chromosomes. Externally, the differences between these two species are subtle: the flowers of the so-called parental species are red or yellow, whereas the flowers of the hybrid can be orange with red spots.

Genetic information alone is insufficient to identify fertile hybrids. Nevertheless, fertile hybrids are now identified with relative ease because, in addition to comparing the number of chromosomes, experts initially examine the most visible aspects of the environments inhabited by the hybrids and the species that gave rise to them. The history of the landscape is then considered by studying the geological maps and climatic variations, which may indicate previous displacements of rocks, past earthquakes or prolonged variations in rainfall or temperature. These historical changes may increase or decrease the proximity of plants or animals with each other, which may or may not influence the formation of new species.

In the case of the above-mentioned orchids, the hybrids inhabited both the *restinga*, the typical environment of *Epidendrum puniceolutem*, and dunes, sites where *E. fulgens* is found. "This versatility suggests that some regions of the genome can be exchanged among these species, giving the hybrid a greater capacity to utilize the habitat," says botanist Fábio Pinheiro, research associate at the São Paulo Botanical Institute. "Natural hybridization is probably one of the explanations for the high degree of diversification that we observe in the genus *Epidendrum*, which includes almost 1,500 species."

Hybrids can arise most easily under conditions that bring together populations of closely related species of plants or animals

As a precaution, in a presentation in May 2009 at the Royal Botanic Gardens, Kew, London, Pinheiro did not mention the number of chromosomes present in the hybrid, for fear of the reaction. "But the orchid specialists at Kew asked, and, when they saw the orchid, they didn't believe it. They said something was wrong, but then they accepted it," Pinheiro says. The prevailing view is that different species do not interbreed naturally and that any hybrids that do form, are sterile. The argument is that the germ cells are not compatible and are thus unable to combine and form viable offspring.

"However, most plants are in fact the result of natural or induced hybridization events between closely related species", says Fábio de Barros, coordinator of the project at the Botanical Institute. Induced hybridization is used to create unique species of orchids and plants used in food production, such as corn and sugar cane. Usually hybrids have an advantage: in the case of food plants, they tend to be more disease resistant and more productive than either of the parent species. "Darwin had already written that hybrids may be sterile or fertile but could not prove it because, at that time, there was no such thing as using molecular markers to identify the genetic signatures of fertile hybrids," says Barros. "Apparently hybridization is quite abundant and seems to have a much larger role in evolution than we ever imagined."

Botanists have already observed other such cases. Orchids of the genus Ophrys, from the Mediterranean region, regularly form hybrids that are highly fertile. Moreover, a cross between two short plants with yellow flowers from Europe and the United States, Senecio squalidus and S. vulgaris, produced a hybrid that attracts more pollinators and could produce more fruit than either of the parental species.

MIXED SPACES

Animals also form fertile hybrids. Thales Freitas, a geneticist at the Federal University of Rio Grande do Sul (UFRGS), observed that two species of subterranean rodents known as tuco-tucos – *Ctenomys minutus*, with 42 to 50 chromosomes, and C. lami, with 54 to 58 chromosomes – are able to cross and sometimes produce fertile offspring. The result depends on the species of the male and female parent: if the female is Ctenomys minutus and the male is Ctenomys lami, the offspring can be fertile, whereas the reverse combination, male Ctenomys minutus crossed with female Ctenomys lami, results in sterile hybrids. A similar situation has been observed for the Atlantic Forest frogs of the genus Phyllomedusa. At the State University of São Paulo (UNESP) and the University of Porto, Portugal, Tuliana Brunes studies the formation of species of Phyllomedusa, the genetic identification of hybrids, and the historical origins of hybrid zones.

The most likely places in which hybrids can arise are in areas where populations of closely related species of plants or animals that were once separated, come into contact. "We have discovered hybrids most frequently in zones of ecological transition, known as ecotones, in which two distinctive types of vegetation mix and facilitate the chances of contact between populations of plants and animals that are otherwise geographically isolated," says Jão Alexandrino of the Federal University of São Paulo (UNIFESP).

Years ago, when he was at the University of California, Berkeley, Alexandrino verified this phenomenon while studying fertile hybrids resulting from the cross between two closely related species of salamanders occurring in riparian woodland habitats in California. Thus far, he, Tuliana, and Celio Haddad (of Unesp) have verified that the resident frogs form hybrids where two types of Atlantic Forest, one more humid and the other drier, are adjacent in the interior of São Paulo State. The hybrids of orchids and tuco-tucos were also found in habitats occupied by groups of species that now coexist. It is probable that this outcome was the result of climatic variations that brought together previously isolated areas or influenced changes in the geographic distributions of certain plants and animals over thousands of years.

A consequence of the processes that lead to the separation of species, thus resulting in crossing or hybridization between closely related species, is that forests with high levels of biodiversity, such as the Atlantic Forest, become "a cauldron of new species in continuous transformation," in the definition of Nuno Ferrand of the University of Porto. "The wealth of biological diversity is not only measured by the number of species but also by the processes that may give rise to new species," said Clarisse Palma da Silva from the Botanical Institute.

The best known mechanism for the formation of new species of animals or plants is the accumulation of genetic mutations in the offspring of the same species. Although it is clear that new species may also result from the clustering of populations of different species that were once separated, not all of these issues have been resolved. "The rules governing the emergence and differentiation of species are not entirely clear because evolution is a continuous process that follows different paths for long periods of time," said Craig Moritz, a biologist at the University of California, Berkeley.


TATIANE NOVISKI/UFRGS

Tuco-tuco: hybrids in the sands of the south

EFFECTS OF ISOLATION

One of the principles that has survived since Darwin is that isolation promotes genetic diversity and the divergence of species over thousands or millions of years. A well-known example is the two species of pit vipers restricted to the islands off the southern coast of São Paulo – *Bothrops insularis*, which only occurs on Queimada Grande Island, and Bothrops alcatraz, which is found only on Alcatrazes Island, located less than 50 km away – that began to differentiate in complete isolation from one another approximately 18,000 years ago (*see* Pesquisa FAPESP *No.132*).

However, much more may still be learned about these species. The research of Ana Carolina Carnaval, a Brazilian biologist now working at City College of New York, indicates that variations in the climate (from dry to wet) and altitude (from zero to 1,600 meters) along a strip of coastline 5,000 km long in the Atlantic Forest favored the isolation, emergence, and development of new species with a greater intensity than in the Amazon, a region where the variations in climate and elevation are not nearly as pronounced. These isolated areas that separate and protect plants and animals form so-called refugia, stretches of forest that survived the intense climatic changes over the millions of years that caused a reduction in nearby forests and the consequent elimination of the animal populations that lived there.

Luciano Beheregaray, a Brazilian biologist who teaches at Flinders University and Macquarie University in Australia, indicated that researchers from the United States, Great Britain and France lead the increasing global scientific efforts in this area, which is called phylogeography and combines genetic, geographical, geological and historical analyses. In the survey by Beheregaray, Brazil, despite its status as the richest country in terms of biodiversity, ranked 15th among the 100 countries examined.

"We can go much further, making more complete analyses of our data, instead of dying on the beach," warned Celio Haddad. "We collected the data, but it is the specialists from other countries that analyzed these data. We should be leaders in this area and not merely towed along." ■

Scientific article

PINHEIRO, F. *et al*. Hybridization and introgression across different ploidy levels in the Neotropical orchids *Epidendrum fulgens* and *E.puniceoluteum*. **Molecular Ecology.** v. 19, No 18. p. 3981-94. 2010.