POLLY WANT A PIGMENT?

Cracking the Chemical Code Behind the Red Colours of Parrots

Text by Dr Kevin McGraw. Photographs by Doug Jansen.

Parrot colours are like no other. The rainbow of hues and fantastic patterns that characterise parrot feathers are unrivalled among any family of animals. Is there a parrot owner out there who hasn't asked the question of their pet: 'Where do you get your remarkable colours from?'

What is even more remarkable is that there was no scientific answer for this question until the dawn of the twenty-first century. Despite the fact that humans have kept parrots as companions for over 2500 years and that chemists have been interested in the nature of parrot colours for over a century, we have only very recently uncovered the molecular secret behind the brilliant colours of parrots.

Some of the earliest work by chemists on animal pigments was done on the molecules that make parrot feathers red, orange and yellow. In 1882, a German scientist named Krukenberg first detected something unique about these pigments. He gave them their own name—**psittacofulvins**—literally 'parrot-pigments'.

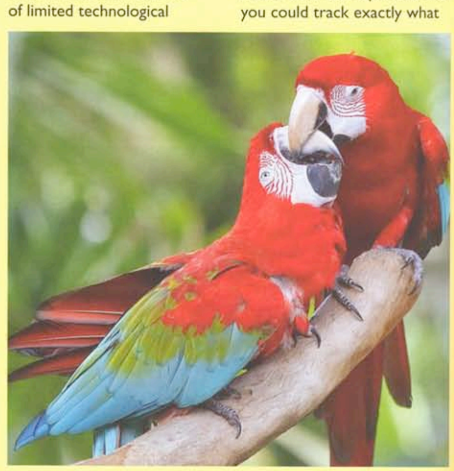
It has long been known that *carotenoid* pigments are responsible for many of the red, orange and yellow colours in nature—the red of tomatoes and flamingos, the orange of carrots and orioles, and the yellow of corn and canaries. However, early on it was noted that carotenoids are not the chemicals that give colour to parrot feathers. Animals must get their carotenoids from

This Eclectus Parrot hen (above) and the Scarlet Macaws (below) display their extensive psittacofulvin-based red plumage.

the foods they eat, since only photosynthetic organisms like plants make carotenoids. As such, the red and yellow feathers of carotenoid-pigmented feathers would moult into duller colours if the birds did not receive sufficient carotenoids in their diets. However, when scientists fed macaws and Budgerigars a variety of diets, including carotenoid-deficient ones, the birds still grew brightly coloured plumage. Parrot owners know that, generally speaking, even parrots on poor diets retain their rich red or yellow plumage.

If the pigments are not carotenoids, then what are they? What makes psittacofulvins different? The first chemical extractions of these molecules a century ago showed that they were lipophilic ('fat-loving') and that they absorbed light at slightly shorter wavelengths than carotenoids. Because of limited technological capabilities at the time, attempts to chemically characterise these interesting pigments stalled. For the remainder of the twentieth century, parrot fanciers were left wondering exactly what beautiful, natural chemicals bestowed colour on their psittacine companions.

Modern advances in analytical chemistry then came to the rescue. In 2001, Italian biochemist Riccardo Stradi used the method of *high-performance liquid chromatography* (or HPLC) to isolate and identify psittacofulvin molecules from the red feather of a Scarlet Macaw. Think of HPLC as a sophisticated napkin absorbing spilled milk from a countertop. If you were to dip just the very edge of the napkin into the milk, you could watch the milk slowly spread its way up the napkin. Now imagine that you have attached a light meter onto the napkin, so that you could track exactly what



The high-performance liquid chromatograph used in this study. I prepared the pigments for analysis by separately pulverising the feathers for each species into a powder with a microniser (a sophisticated grinder) and adding an organic solvent to extract out the pigments. The solvent samples were then added to the revolving sample tray and injected with a mechanical-arm injector into the HPLC column (contained within the heater shown in the photograph, which is used to warm the solvent and make the molecules move faster through the column). As molecules eluted from (came off) the column, they were channelled via plastic tubing into the light-absorbance detector (called a photodiode array detector), which determined the light-absorbance properties of each type of molecule present in the feathers and sent this information to a computer (not shown) for analysis.

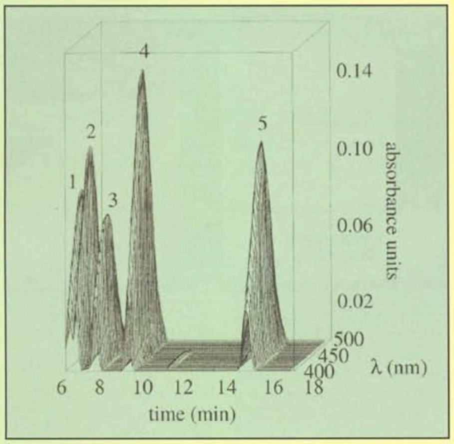
molecules were travelling up it (based on what wavelengths of light they were absorbing—the definition of colour) and exactly how far they travelled. This is the essence of HPLC. Instead of a napkin, you use a tightly packed column of silica beads to which molecules bind differentially. By first dissolving these molecules in a solution that is injected into the column, you can separate them all out under high pressure and measure their light-absorbance capabilities.

Using HPLC, Dr Stradi recognised five unique and colourful molecules that were present in red Scarlet Macaw plumage, all of which fell into a class of pigments known as *polyenal lipochromes*. This means that they all are lipid-soluble and share the molecular feature of having a linear chain of carbon and hydrogen atoms, accompanied by a single oxygen atom at one end of the molecule. He had cracked the chemical code to red psittacine colouration!

Perhaps the most exciting aspect of this work was that these molecules had never before been described in any living or non-living thing in the universe. The pigments and colours of parrots are thus truly distinct from the colours of all other birds and animals!

With this basic foundation of information, one of the next logical questions to ask is: What about the rest of the parrots? Nearly 300 species of parrots have some red, orange or yellow in their feathers. Do they use these same five molecules, occurring in the same ratios, perhaps, in their feathers?

This was the aim of my recent study, done in collaboration with Mary Nogare, an aviculturist from Washington in the USA. Mary in fact initiated the study, as she has had a life-long fascination with parrots and a knack for asking really insightful scientific questions about her birds. Mary and I have never even met in person! She found me and my research expertise on the World Wide Web, and knowing that I used HPLC to study bright carotenoid-based colours of songbirds—including finches, sparrows, and warblers—Mary



A representative three-dimensional chromatogram obtained from our HPLC analyses of the parrot feather pigments. This is the data collected directly and sent to the computer by the photodiode array detector. Tall peaks represent the molecules that came off the column. Along the horizontal x-axis is time, so you can see how the five different (numbered) pigments were separated out by the column over a period of around 10 minutes. The height of these peaks (measured along the vertical y-axis) is an indicator of how much of each molecule was present in the sample. Species of parrots that were redder had higher peaks (sum totalled for all peaks) than species with less-red feathers. Notice that the individual psittacofulvin types certainly differed in concentration in this sample, but the relative amounts of these pigments did not seem to show any pattern with respect to the sex of the parrot for which we were analysing feather pigments, the species of parrot, or feather-colour intensity. Along the diagonal z-axis are the wavelengths at which these molecules absorbed light. Note that each peak has a single apex, which shows that it absorbs light maximally at one wavelength (around 425–450nm).

suspected that we could successfully extend Dr Stradi's work on psittacofulvins in Scarlet Macaw feathers to many other parrots that display some red, orange or pink in their feathers.

The goal of our research was in place, but we still needed birds and their feathers to study. Loro Parque aside, there are few facilities in the world where one has access to dozens and dozens of colourful parrot species. Also, funds were limited for this project that was to be done at such an early stage of my scientific career (I was approximately halfway through my 4.5-year PhD program when Mary and I initiated this line of work), so we enlisted the help of many proud pet-parrot owners across the USA, as well as various zoos, museums and pet stores. We asked the owners or caretakers of live birds to save feathers for us when they were shed onto cage floors, and had museum curators pluck a few colourful feathers from their parrot specimens. In all, we assembled red, orange and pink feathers from 44 species spanning all of the major groups of parrots, including cockatoos, lories, lorikeets, macaws, parakeets, Amazons, rosellas and fig-parrots. This sample also included one of the more primitive of parrots, the Pesquet's Parrot (or Vulturine Parrot) from New Guinea.

Our HPLC analyses of feather pigments showed that the same five psittacofulvins were responsible for the range of reddish and orangish hues in all of the species in our study. This means that not only have novel pigments been described in colourful parrots, but this same mechanism is likely responsible for the red colours of all parrots. As an evolutionary biologist, this intrigued me: Why have only parrots stumbled across this novelty?

We went on to begin to answer this question by searching for the source of these molecules. Presumably they were not diet-derived, as discussed previously, and sure enough we have not found these pigments in any parrot foods. We tested samples of blood taken from parrots while their colourful feathers were growing, and again psittacofulvins were not present. (The bloodstream is the route via which they would transport pigments acquired from the diet to peripheral sites in the body for colouration.) This suggests that parrots make these pigments internally, and do so at the actual sites for colouration—the feathers. We have ideas about how they may accomplish this feat (for example, by metabolising fatty acids), but as with most scientific endeavours our research has raised about as many questions for future study as it has answers. Our work is now cut out for us.

Many of you may wonder: What about the other colours of parrots, such as the greens and blues and yellows? How do Budgerigars and Amazons make these colours, for example? Work done on the greens and blues of parrots in the 1970s by a Danish ornithologist named Jan Dyck

Military Macaws (above) and Red-fronted Macaw (above right) display green colours partly generated as a result of light-scattering by feather-tissue components creating blue colours and yellow pigments in the feather.

showed that blue colours are a structural phenomenon—the result of light-scattering by feather-tissue components like keratin. Green colours are also generated in part by this structural mechanism, but green feathers also contain a yellow pigment (yellow and blue makes green!). We set out to identify the yellow pigments in our study, but had quite a bit of trouble with them. It is clear that they too are not carotenoids and that they exhibit very similar properties to the red psittacofulvins we have studied, but up to this point we have been unable to completely characterise them for various analytical reasons (the pigments do not clearly elute and separate from the column). The nature of these yellow pigments will continue to be a high priority in our ongoing work.

Another facet of our study will be to investigate the fluorescent nature of some yellow-coloured regions of plumage in parrots (eg in Budgerigars and caiques). Do these feathers fluoresce due to a unique pigment, or is there some structural component to the feather tissue itself (perhaps how the pigment is bound to the keratin) that allows for its fluorescent appearance? Stay tuned.

So the next time you look at your colourful parrot, think of sophisticated napkins, polyenal lipochromes, and the exciting work that remains to be done on the enzymes that help parrots make these pigments as well as the genes that ultimately control the ability of parrots—and parrots only—to make them.

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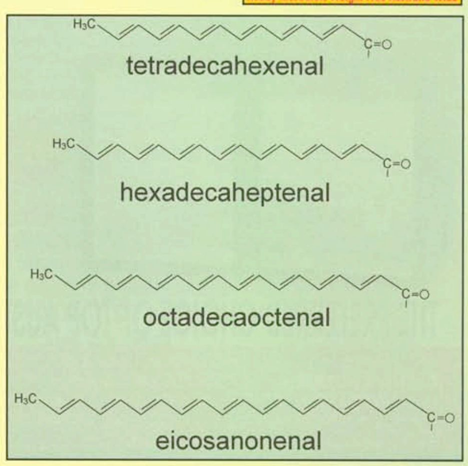

The molecular structure of the newly described pigments— the psittacofulvins—in parrot feathers. It is the long chain of carbon atoms in conjugation with one another (alternating between single C-C bonds and double C=C bonds), known as the chromophore, that allows these molecules to give colour to tissues like feathers. Pigments absorb light, and this bond system allows psittacofulvins to absorb short-wave light (eg blue and green), thus leaving long-wave light like yellow, orange and red to be reflected and seen by our eyes!