Microscopic and chemical investigations of canary feathers

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The variety of canary colours has long held a great fascination for me. That is why I have been breeding different coloured canaries for more than 30 years; that is why the desire to learn more about the laws of heredity has grown in the last few years. I wanted to know more about the colours of the canaries and how they come about.

The formation of melanin has been and is being researched in depth and comprehensively. Today we know that there are eu- and phaeomelanins in nature, we know how they are formed and stored, and we know many genes that control these processes. To gain this knowledge, many chemical, biochemical and electron-microscopic investigations are necessary. The results are published in the respective specialist literature, the examination protocols are made available to interested parties and can be found in the references of scientific papers [1].

Unfortunately, canaries have never been used to study melanogenesis. Nevertheless, the following statements can be found in almost every canary book and in many publications:

Initial situation

The following statements can be found in almost every canary book and in many publications:

- The colouration of canaries can be produced by eu- and phaeomelanins and by lipochromes.
- Phaeomelanins are deposited more on the feather edge, eumelanin mainly in the centre of the feather, on or in the quill.
- Some canary colourings are said to have only one particular melanin form – only eumelanin or only phaeomelanin or only certain proportions of both.
- The round, red to light brown phaeomelanin granules are readily soluble in alkalis and the elongated oval, black and brown eumelanin granules are sparingly soluble in alkalis.
- The melanins are only present in reduced numbers, the original melanin colours are "diluted".
- Melanins have changed or transformed from one form to another.
- Certain canary colours are attributed to an altered cell structure of the feather and are then called structural colours.

Etc. etc.

Whether these statements actually apply to canaries has not been investigated; rather, they are analogies from the findings of other bird species or even animal genera.

In order to be able to make these statements, a large number of chemical, biochemical and electron-microscopic examinations are necessary. From time to time one can find references in canary literature or in articles to investigations that have been carried out on the cause of the feather colouration in question. Unfortunately, there is no reference to the source of this information. As these examinations are very complex and expensive, it is to be expected that the results will also be published in the respective specialist literature and that the examination protocols will be made accessible to interested parties.

However, in my search for these sources I have had **no success** so far, and this despite years of searching. If there are references, they refer to findings from studies of other bird species, most of which are not closely related to the canary. Melanin formation has been researched very deeply and extensively. Canary feathers were only very rarely or never used for this purpose.

Until today, canary breeders have helped themselves by transferring findings from scientific research on other animals and birds to the canaries. This is a legitimate means to describe certain facts more profoundly. However, it bears the danger that facts can simply be misinterpreted.

Conclusion: There are no publications studies on colour canary that can **prove** the above statements! Therefore, I must assume that many statements about canary colours are unproven assertions or hypotheses.

Contradictory statements

In ornithological literature, the interaction of melanin-containing medullary cells and lipochrome-containing cortical cells to produce different colours is mentioned. As a striking example, the development of the different colours in the budgerigar (*Melopsittacus undulatus*) is very often cited. Usually the drawing by Fritz Franz (Figure 1) is used and his statement is interpreted very freely. The origin of the budgerigar colours is then explained as shown in pictures 2 to 5.

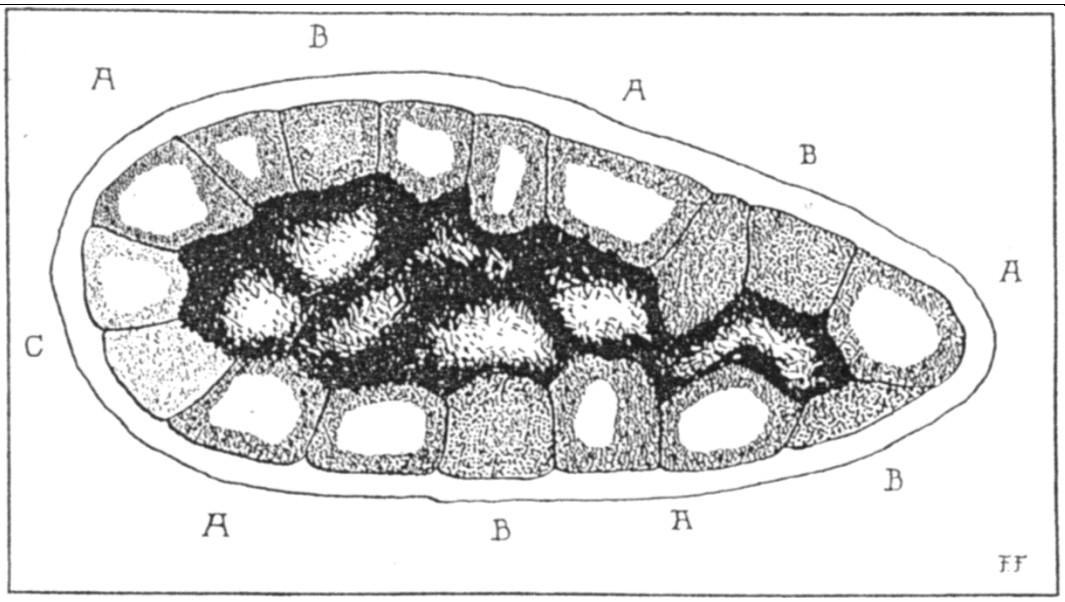

Figure 1:
Halmahera pitta (*Pitta maxima*) cross-section through a wing feather branch (ramus) impregnated with stained ether collodion. From outside to inside: Cortex layer, box cell layer, pith cells filled with melanin (original drawing: F. Frank 1939).

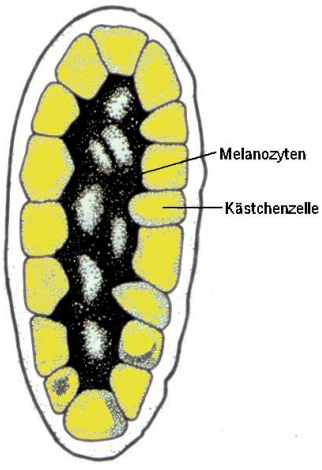

Figure 2:
Green budgerigar: melanin-containing medullary cells; yellow bark cells

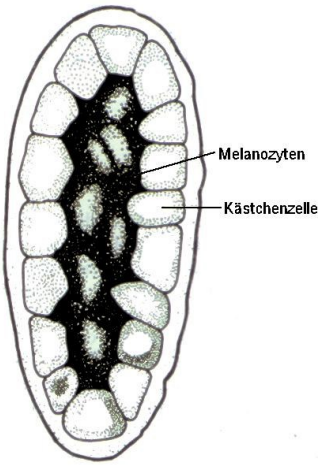

Figure 3:
Blue budgerigar: melanin-containing medullary cells; colourless cortical cells

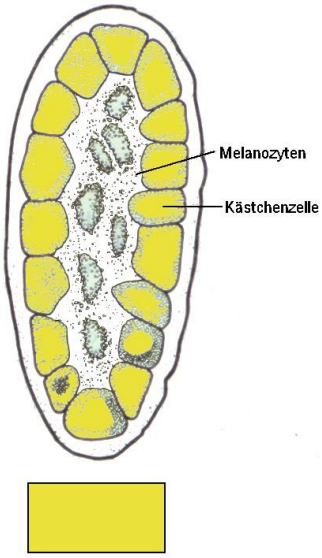

Figure 4:
Yellow budgerigar: colourless medullary cells; yellow cortical cells

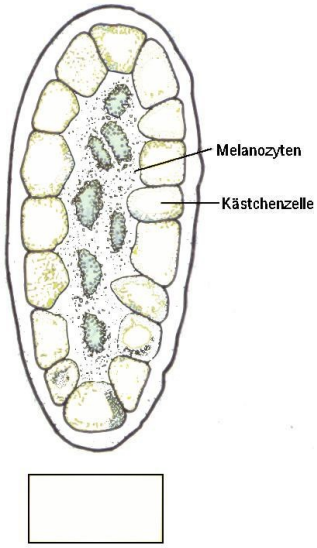

Figure 5:
White budgerigar: colourless medullary and bark cells

Obviously, no one has ever verified this statement, otherwise it would have been recognised quite quickly that there are no melanin-containing medullary cells in any feather. I have examined a whole series of feathers of different birds with a light microscope and have never been able to find melanin-containing cells in the medullary canal of the feathers – not even in the budgerigar!

Figure 6: black redstart *(Phoenicurus ochruros)*

Figure 7: carrion crow *(Corvus corone)*

Figure 8: Pacific parrotlet *(Forpus coelestis)*

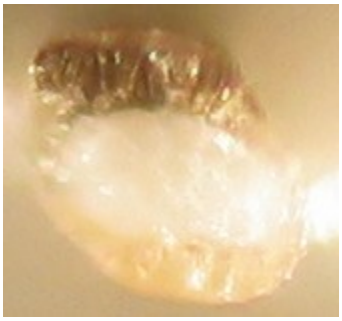

Figure 9: zebra finch *(Taeniopygia guttata)*

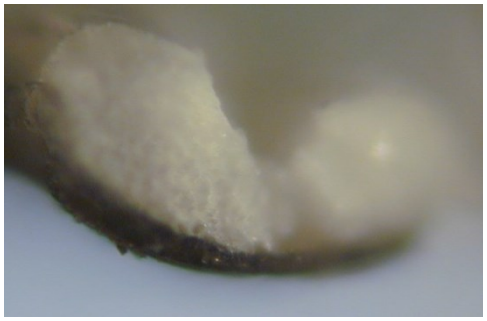

Figure 10: king quail *(Synoicus chinensis)*

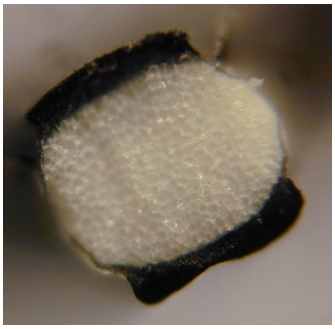

Figure 11: Budgerigar grey

It is undisputed that there are two different types of melanin, eu- and phaeomelanin. So let us assume that the colouration of melanin-containing canaries is caused by eu- and pheaomelanins.

That eu- and phaeomelanins can be distinguished by a certain **granule shape** (elongated eumelanins and round phaeomelanins) is, however, still **disputed** among scientists. There are scientists who claim to have found round eumelanins and elongated phaeomelanins.

Regardless of this, canary literature refers to the elongated, rod-shaped granular form of the eumelanins and the round shape of the phaeomelanins.

It is undisputed that – from a chemical point of view – the phaeomelanins are distinguished from the eumelanins by a higher proportion of the sulphur-containing amino acid cytesin.

Eu- and phaeomelanins are also said to be distinguishable by their solubility in alkalis. We can also read this from time to time in canary literature. However, we do not read those other researchers have found that **light melanins are easier to dissolve than dark ones** – regardless of whether they are eu- or phaeomelanins!

Under the electron microscope the melanin grains can be made visible, but unfortunately it is not yet technically possible to determine the actual colour – the photos are black and white. Since the granular form does not help either, we do **not know** whether and in what quantity Eu- and/or Phaeomelanin are present in the canary feathers of the individual colour varieties.

Melanin grains are stored in the nib in a certain **density**. A lightening of the melanin colour can have many causes. A reduced density is only **one** possible way. However, no one has yet determined this density. Thus, a reduced density (melanin dilution) could not be proven yet either.

A cause could also be, for example, reduced melanin storage in the melanosomes. Which of these causes actually occurs in the colour varieties concerned has not yet been investigated.

If this is the case, I have to do my own investigations and began with the chemical examination of melanin-containing canary feathers.

Chemical investigations

In the literature it is described that eumelanin is not soluble or only very slightly soluble in 45 % lye. Phaeomelanin, on the other hand, already dissolves in 2 % lye.

That had to be comprehensible. I therefore procured 45% and 2% potash lye, back feathers from black, brown, agate, satinette and phaeo canaries and placed one feather in each test tube with the 45% potash lye, another feather with the 2% potash lye.

I left the feathers in the lye until the beginning of their decomposition. A change in colour of the lye could not be observed in any case! Not even in the feathers of phaeo-canaries! Heating the feathers floating in the lye to the boiling point did not give any other result.

Does this mean that there is not easily soluble phaeomelanin in the canary feather?

For a further comparison, I repeated the experiment with the reddish-brown flank feathers of wild zebra finches (*Taeniopygia guttata*).

After a few seconds the potash lye turned yellow, after a few minutes it turned orange! Both with 45 % and 2 % caustic potash solution. After longer exposure to the lye, the flank feathers of the Zebra Finch almost completely discoloured.

In the literature, the easily soluble phaeomelanin is held responsible for the reddish-brown colouration of the zebra finch flanks!

Such a simple experiment – which can easily be carried out by anyone – has obviously never been done before, because then justified doubts would have arisen as to whether there is phaeomelanin in the canary feather at all. Only later did I learn from studying various literature that scientists are not at all in agreement about the solubility of the two types of melanin. For example, light melanins are generally more soluble than dark melanins, regardless of whether they are eu- or phaeomelanins.

Having become completely unsure, I remembered that eumelanins and phaeomelanins can also be distinguished in their granular form. To do this, electron microscopic images are necessary, but who has the means to do this? I tried to get more information with a light microscope.

Light microscopic investigations

I acquired – thanks to the support of the teaching staff of the Dresden-Plauen grammar school – a normal microscope, an electronic camera with the corresponding microscope tube and a monitor (screen diagonal 33 cm).

To be able to determine the relative magnification of the images produced, I used the following procedure:

I used human scalp hair for size comparison. I set the thickness of the hair to an average value of 0.06 mm. On the screen, the width of the hair, and thus the relative magnification of the individual eyepieces, could be measured.

Examination material

The feathers to be examined come from the canaries from my own breeding and from birds of friendly breeders. I attached importance to yellow- and white-grounded birds, because I think that feathers of these birds make the melanin most visible without falsification. Only for comparison, feathers of red-ground canaries were used.

The melanin canaries existing today probably only very rarely correspond to the original colour mutations that occurred. Even today's black canaries – from which some colour mutations originated – have hardly any relation to the original black-brown melanin colouration of the wild canary. However, in order to be able to draw comparisons between the original colouration and today's changed colouration, I used feathers from the European Serin (*S. serinus*).

In this first study, feathers from the European Serin and from coloured canaries with the melanin colours black, brown, agate, isabel, (brown)satinette, black, agate and satinette-opal, black onyx as well as brightened in red and yellow were used. Feathers from other bird species were also used for further comparison.

In order to be able to make comparisons between the different colours, only back feathers and feathers of the large plumage (tail feathers and/or wing feathers) were used. Of the back feathers, those with a clear melanin streak in the centre of the feathers were selected.

The following feather parts were examined:

- Down branches and down rays of the base down of the dorsal feather.
- Quills of the main feather and the dorsal feathers in top view and in cross section.
- Lipochrome-containing and melanin-containing feather branches of the dorsal feathers in top view and in cross section

Examination methods

The above-mentioned feather parts were taken individually from the respective feather, placed on a microscope slide and fixed with a cover slip. For individual smallest feather parts and for a magnification of approx. 1300 x, fixing oil was used in some cases.

Cross-sections through quills and feather branches could only be made using sharp scissors (bruise possible), as attempts at micro-sections failed due to the brittleness of the feather parts (splintering).

All objects to be examined were not coloured.

The objects were observed in transmitted light and/or reflected light; cross-sections only in reflected light. The setting with the greatest recognisability of details was photographed.

The photos were transferred to a computer and post-processed if necessary. Only cut-outs were made, or backgrounds were edited to facilitate the greatest possible expression. Nothing was changed in the colour or contrast of the pictures or parts of the pictures.

Unfortunately, the technical requirements can only be described as quite simple. The placement and processing of the finest feather parts (barely visible to the naked eye) on the slide proved to be quite difficult and required a high degree of patience and fine motor skills. Therefore, of the several hundred images, only a small number are suitable and meaningful for publication.

Down

The downy branches of the dorsal feathers consist of cells arranged linearly one behind the other, which have visibly deposited melanin. The cell walls are more pigmented (Fig. 12).

The down rays are attached to the down branch by means of a broad base. From this flattened and colourless base, the actual round part of the dune ray emerges, which is clearly divided into many sections.

Each section thickens at its end to begin again more narrowly with the next section. This creates "nodes" that contain melanins alone. The regular succession of these segments creates a "blade of grass" structure (Fig. 13).

The colouring of the nodes is a deep black in the European Serin, the black canary and the agate canary, and a greyish brown in brown and isabel birds. In agate and isabel birds, the coloured extent is reduced, and colourless or almost colourless nodes occur more or less frequently.

In the cases I have examined, brown, agate and isabel canaries have crown-like tips at the thickened end of the nodes.

Lipochrome colours are not detectable in all parts of the downs.

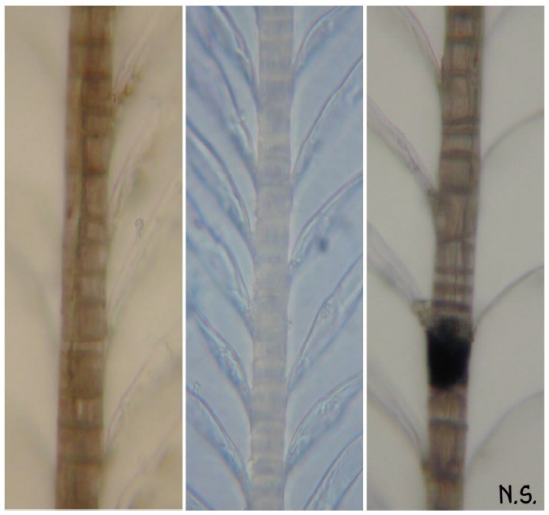

Figure 12: Branches of down 1,300x magnification; from left to right: black canary, isabel bird, agate opal bird

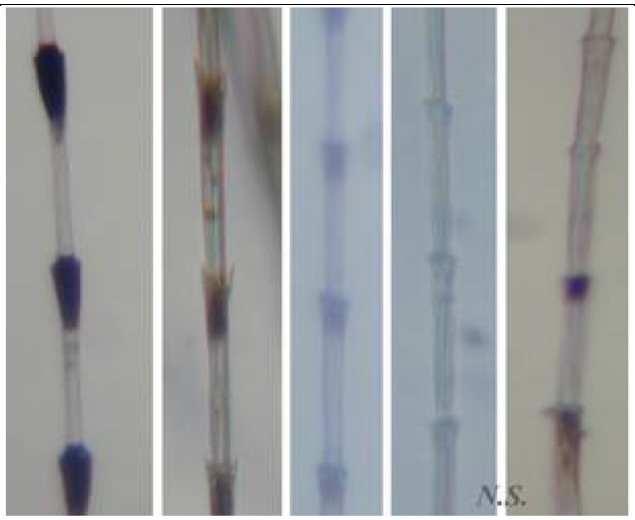

Figure 13: Down rays 1,300x magnification; from left to right: black-yellow; brown-yellow; brightened yellow; isabel yellow; agate opal

Quills

In most of the melanin canaries the feather upper side are darker than the underside. This is also reflected in the quills. In unmutated melanin birds (here serins and black yellow canaries) the feather cells of the quill upper sides cannot be made visible (too much melanin storage?). Despite recognisable structures within the colouration, a differentiation of melanin granules is not possible even at 1300x magnification (Fig. 14).

The cortical cells of the keels are mostly pentagonal to hexagonal and give the impression of a snakeskin. Depending on the colour of the canaries, the area between the cell walls is lighter than the clearly darker cell walls. Depending on the size of the keel diameter, more or less cells of approximately the same size form the keel.

The bark cells of the keels are mostly pentagonal to hexagonal and give the impression of a snakeskin. The area between the cell walls is, depending on the colour of the canaries, lighter than the clearly darker cell walls. Depending on the size of the keel diameter, more or fewer cells of approximately the same size form the keel.

The cross-sections of all keels – including those of opal birds – all have the same characteristics. The horn-coloured medullary cells make up the larger part. The bark of the upper and lower sides is thicker than the sides. In the cross-section of the bark, no structures can be seen or only structures can be seen in the bark in cross-section. However, the colour of the melanin is clearly discernible (Fig. 15; cf. also Figs. 6 to 11).

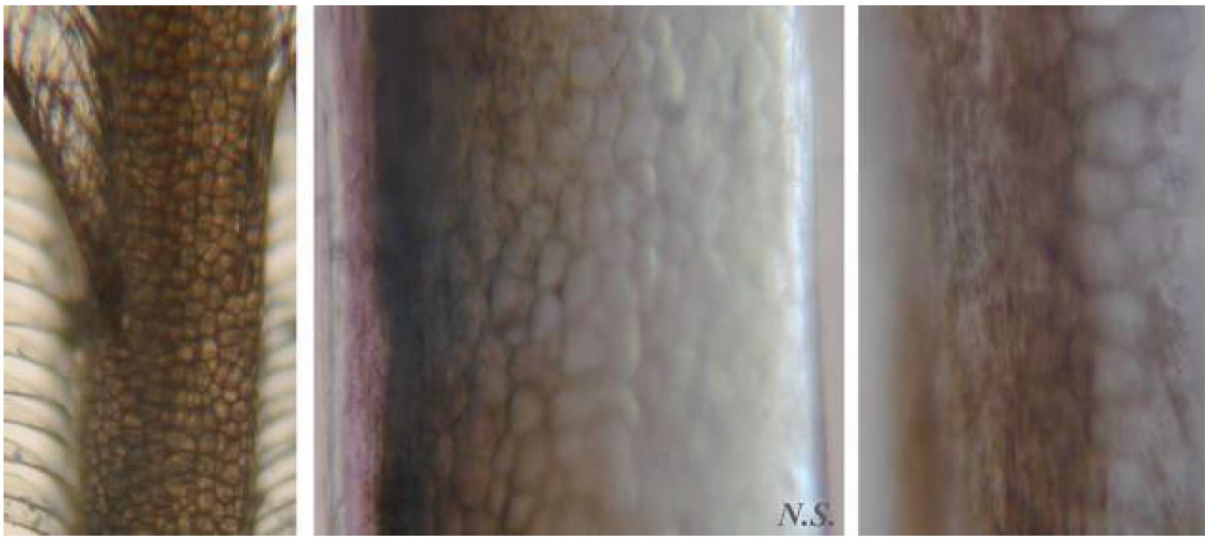

Fig. 14: Quills of the large feather 380-, 750- and 1,300-fold magnification.
from left to right: Cell structure of the quill (brown bird); quill black bird; quill brown bird (in the two pictures on the right, the more pigmented side is the upper side of the feather).

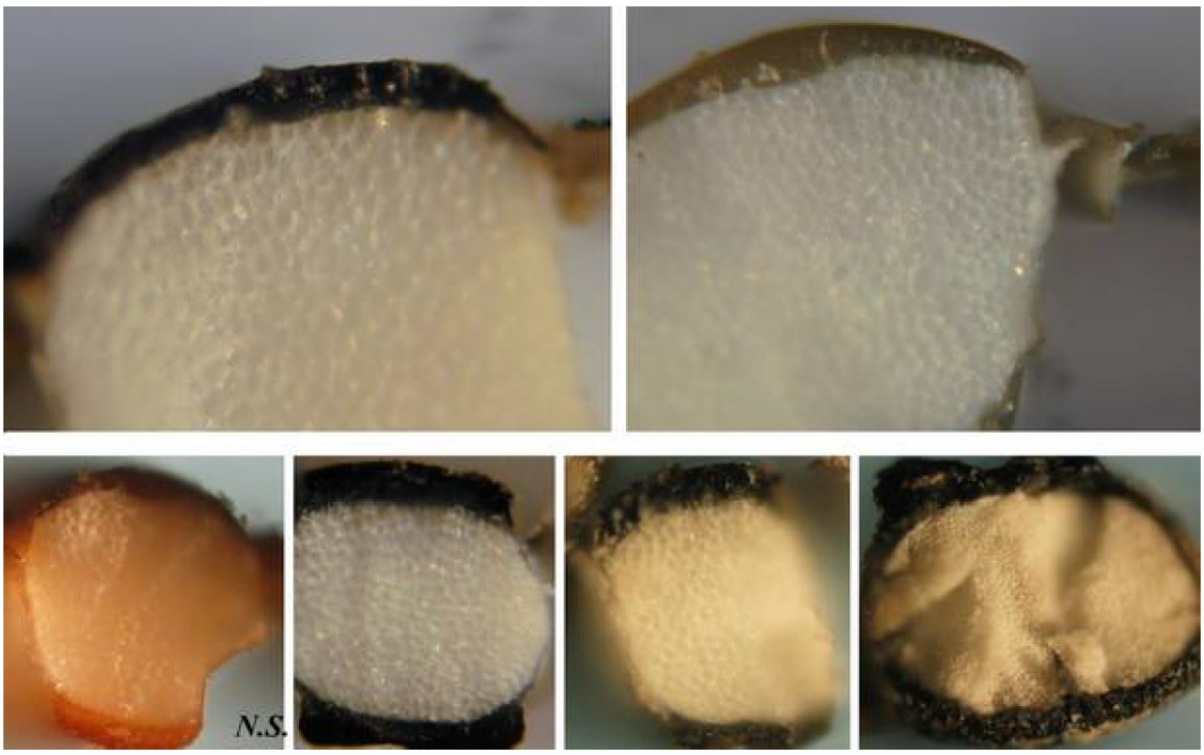

Fig. 15: Cross-sections of quills of the large plumage of various bird species.
top left: European Serin, bark of upper feather;
top right: Isabelline Yellow, bark of upper feather.
Bottom row cross-sections in comparison;
from left to right: black redstart, budgie grey, Pacific parrotlet, carrion crow.

Feather branches and feather rays

The hooked and arched rays of the large feathers of melanin-containing birds show a differently strong, but always clearly visible melanin colouration, which is naturally absent in the lightened birds. Lipochrome colouration could not be detected in any case (Fig. 16).

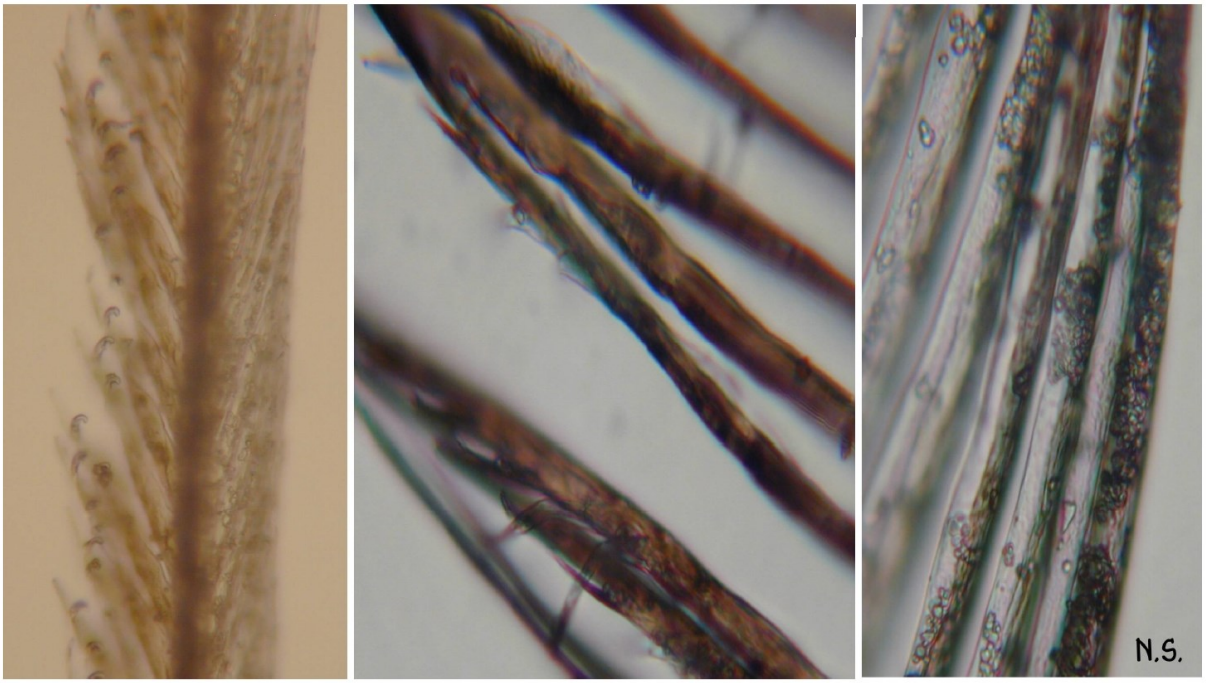

Fig. 16: left: Feather branch with hook and bow rays European Serin (110x magnification);
centre: Serin hook rays;
right: black canary bow rays (both 1,300x magnification).

In poor representatives of the black and agate birds, brown (phaeo)melanin is mixed in with individual feather branches. This undesirable brown component is usually visible in the surface melanin and has a reddish-brown tint. Whether it is really phaeomelanin I could not determine with my methods.

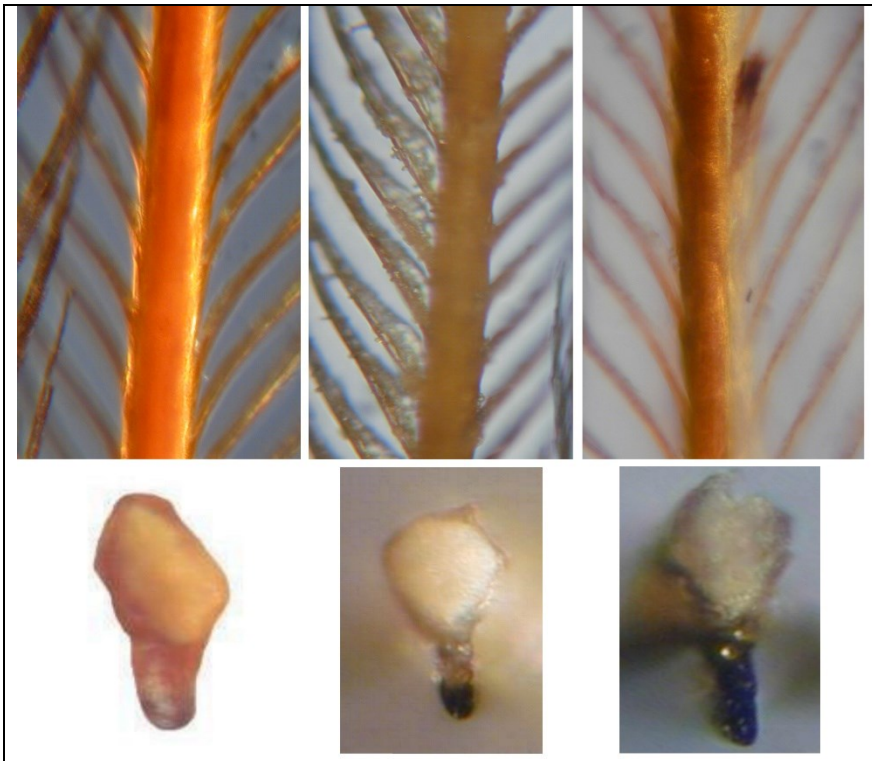

Fig. 17: Dorsal feather branches of a black red bird (750x magnification).
from left to right: lipochrome-, brown(phaeo)containing-, black-containing feather branch.

Near the base, the feather branches have stored a relatively large amount of melanin. Towards the tips of the branches (outside of the feathers) the concentration of melanin decreases and the lipochrome increases. An accumulation of melanin at the tip of the branch could not be observed in any case – not even in the European Serin.

Lipochromes are only stored in the bark cells of feather branches. This applies to both brightened and melanin canaries (Fig. 17).

The feather rays of the lipochrome-containing feather branches have at best only deposited lipochromes at the base. The feather rays are therefore only involved to a small extent in the colouration of the feather.

The special case Opal

In classical melanin canaries, the bark cells of the upper side feathers are more strongly coloured than the bark cells of the undersides of the feathers.

In contrast, in the melanin-containing feather parts of opal birds, large punctiform melanin accumulations are found. Whether these are macromelanosomes (melanosome = pigment granules) or a concentration of many small melanosomes cannot be determined by light microscopy.

These "melanin agglomerations" are mainly found in the bark of the undersides of the feathers and only a few in the upper the bark of the upper feathers. The spaces between the melanin balls are only slightly or not at all coloured with melanin. not coloured with melanin.

I could not detect any change in the feather structure, i.e., the feather cells.

In the course of my breeding experiments, I also achieved Satinette Opal birds. Visually, these do not show any melanin in the phenotype (not even in the under feathers), have bright red eyes and thus resemble "Inos". Nevertheless, I found similar melanin aggregations in the feathers of these birds as in the black and agate opal birds. However, the number of these melanin accumulations is much smaller, and they have a light brown colouration (Fig. 18).

The examined feathers of the Onyx canaries also showed melanin clusters. However, these are smaller than those of the opal birds and are found both in the bark of the upper side and in the bark of the underside of the feathers. Between these melanin concentrations, melanins are interspersed, as can also be seen in the classical melanin canaries.

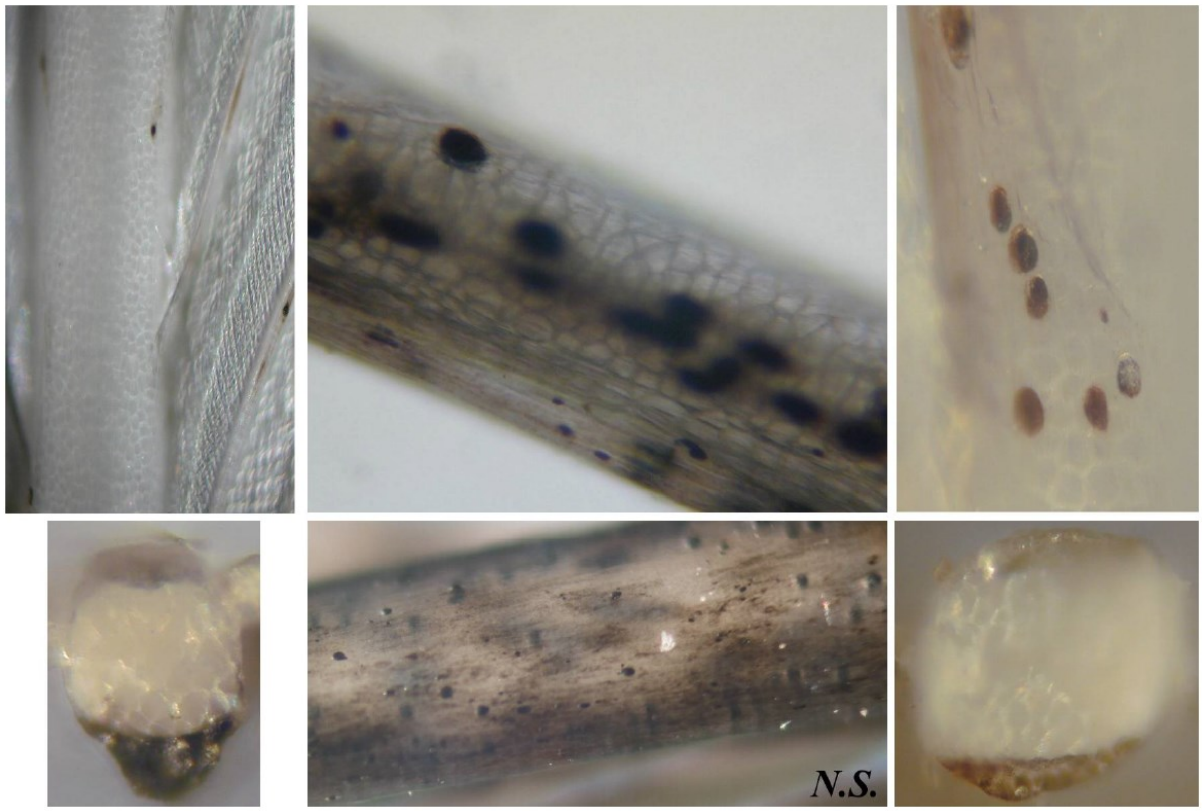

Fig. 18: Opal and onyx feathers
top from left to right: dorsal feathers of agate opal recessive white, upper side of feathers; same underside of feathers; satinette opal yellow
below from left to right: cross-section through keel of a back cover feather agate-opal recessive white; upper side of a back cover feather black onyx; cross-section back cover feather satinette opal yellow.

Generally valid findings of the investigations

1. **Lipochrome staining** occurs increasingly in the **bark cells**. In some parts of the feathers, they occur together with melanins.
2. **Melanin colouration** could basically only be found in the **bark cells** of the quills and branches. The medullary cells of the quills and branches have a horn-coloured staining and do not show lipochrome staining.
3. **Melanin pigment grains** are **not visible** visually and by light microscopy. Consequently, a distinction from elongated eumelanin granules and/or round phaeomelanin granules is not possible. However, it is possible to draw conclusions as to whether the melanin is black or brown on the basis of the visible colouration.
4. The amount of **melanin present cannot be determined**. At best, the depth of colour – black or grey, brown or light brown ... – can be determined.
5. Relatively large **melanin agglomerations** are clearly visible in **opal** and **onyx birds**. These agglomerations are found more on the underside of the feathers in opal birds. A change in the feather structure (structure of the feather cells) could not be determined.

Conclusions

- The interaction of melanin-containing medullary cells and lipochrome-containing bark cells to produce different colours is mentioned in the ornithological literature [2]. As a striking example, the development of different colours in the budgerigar (*Melopsittacus undulatus*) is often cited as. According to this, melanin-containing (black) medullary cells and yellow-coloured bark cells are supposed to produce the green of the budgerigar. Depending on the omission of one and/or the other colour component in the medulla and/or bark, blue, yellow and white budgerigar colours should be produced.

  Obviously, no one has ever verified this statement, otherwise it would have been realised quite quickly that there are no melanin-containing pith cells in any feather. I have examined a whole series of feathers of different birds with a light microscope and have never been able to find melanin-containing medullary cells in the feathers – not even in the budgerigar!
- As the type of melanin can neither be determined visually nor by means of a light microscope, I do not think it is accurate to speak of eu- and phaeomelanin. We should speak in general terms of black, brown, reddish-brown ... melanin.
- The cause for our birds with "diluted" melanin – Agate and Isabell – can be very different. On the one hand, the number (density) of the melanin grains can be reduced – but this cannot be determined by light microscopy – on the other hand, the tint of the melanin grains can be lightened (anthracite, grey, light brown ...).
- In connection with opal birds, one should not speak of a "structural colour", as a structural change (change in feather structure) has not been observed.

  Visually, the melanin concentration – with the melanin-free areas in between – causes the same as an even distribution of a smaller number of "normal" melanin grains – namely a melanin brightening. Black melanin is "diluted" to grey, grey melanin to light grey, brown melanin to light brown.

  The feather cells on the upper side of the feather that have become low in melanin thus lie on a dark background and thus reflect an increased proportion of blue light (similar to a mirror or a water surface). This results in the blue shimmer of grey to light grey melanins on the upper side of the feathers. This has nothing to do with the "Tyndall effect" often used in this context in opal canaries. This effect of light scattering occurs in colloidal solutions, i.e., solutions of finely distributed particles in an otherwise colourless medium. We know this, for example, as sun rays or headlight cones in fog.

It is very surprising to me that no microscopic pictures of the colouration of canary feathers have been published so far, although some statements can already be made with similarly primitive means as I used. I can only hope that I will set a ball rolling with this publication and that other breeding friends or even scientists will turn their attention to this subject.

Literature

[1] **Onsman, I.:** In Quest of Events Leading to Pigmentary Disorders in Several Mutants of the Budgerigar (Melopsittacus undulates); a Light- and Electronmicroscopical Survey. MUTAVI Research & Advies Groep.

[2] **Frank, F.:** Die Färbung der Vogelfeder durch Pigment und Struktur. At: Journal für Ornithologie, Heft 3, 1939.