SNIFFER RESEARCH

A look into the future: **THE EGG SEX SNIFFER** Egg odour reveals embryo sex 1st day of incubation

Science continues to research all aspects of birds, also in fields which have not been explored yet. The research where this article is about is BREAKING and might be the onset of the ending of the cruel practice of shredding or gassing male chicks from commercial layer breeds.

Thinking further: maby we as fanciers might one day in future decide ourselves how many male chicks we want...

Breeding year 2014 was characterised by a huge quantity of male chickens, leaving everybody with a surplus of roosters. This is annoying on the one hand, and on the other hand you had plenty of choice to be very selective on the best of the best cock, who will eventually guarantee your improvements this breeding year 2015.

Science never did particular research on the volatile compounds (odours, smells) which bird's eggs emit although it is known eggs do smell and they attract predators and parasites.

The smell of eggs might be part of the interaction between parent and embryo and embryo to embryo before movement and vocals come in during development.

There are breeders who talk to the eggs during incubation in the bator, its not weird at all since in the last part of incubation the embryos do react on influences from outside. You can even see this when you candle the eggs at day 5-7; the embryos can go wild.

Egg-smells can be used as communication before the embryo can talk or move which tells the status of development by which synchronised hatching is possible plus the parent is encouraged not to leave the eggs. The embryo-parent bonding by the talking embryo can only be established a few days before hatching so the smell of the eggs is a way of bonding before that time, which is much longer. The smells tell the parent the developmental status of the embryo and it might provoke fysical and behavioral changes of the parent to brood the best way (for example: nitric oxide from the developing embryo stimulates development of the

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brood-patch of the parent) or abandon the nest.

There is no information available about the compounds of the odours from developing embryos. In order to find out the role of egg-smells, these compounds had to be determined, which is done in this research. There has been done some research on the avian chemical ecology but it is a very young research field. For this research were Japanese quail eggs used which are very much related to our chickens.

Aim was to find out if the composition of the odours tells something about egg fertility, embryo development and embryo sex which is interesting in the interaction between parent-embryo and the embryos amongst eachother.

During the first stages of embryo development there is no difference between males and females and there is reason to assume there is a difference in egg compostion between fertile and infertile eggs. So all eggs should have the same odours.

When the development of the embryos progresses it can be expected the odour compositon changes based on fertile and infertile eggs of which the fertile eggs may change compostion due to the developing embryo, its growth rate and later the sex differences. The researchers made therefore two predictions, the first:

- there will be an interaction between fertility and development vs egg odour composition in which the compo will not differ between fertile and infertile, but there IS a difference later in development; and second:

- that there will be an interaction between the sex of the embryo and its developmental stage with no sex differences during the early stage of incubation but WITH differences between the eggs in later stages between male and female embryos.

## How to find out?

You can find the methods and materials in the research paper. In short: fresh quail eggs were stored for max. 5 days in fridge at 5C. Then cleaned, and put in Brinsea Octagon 20 Advance inci at 37.5C and 60% humidity for 8 days (of the total of 17 for quails). A few days after the 8th day the embryos start to develop hearing and feeling vibrations, therefore day 8 is choosen because they are still too premature.

The collection of odours was done in a special set-up on day 1 and day 8. Analysis of the chemical compounds was done in a gass chromatography-mass spectrometry thingi.

Sexing was done on day 8, on molecular level (PCR)based on sex-linked genes. Then a statistic was set-up because you can't measure if you don't know Zero. This was done for day 1 and day 8 of incubation. They visualised the odour compositions using canonical analyses of principal coordinates, which is multivariate. Via Pearson's correlations they standardised the data for each compound to indicate the relative contribution of each, in the two groups. This is pretty much bla bla language already, so I skip this part.

# Okay, what did the researchers find?

The measured compounds were set out on two axis. WHAT chemical compounds they found was not yet known when they started this research, therefore this paper is unique in its kind.

They found 3 which could be identified and 2 which couldn't. The eggs showed a change in odour composition between day 1 and 8.

On day 1 there was no difference in compounds between fertile and infertile eggs, but there was a highly significant difference on day 8. (see paper for the compounds).

For the sexing test, there was a significant change in odour composition between day 1 and day 8 for eggs containing both male and female embryos. On day 1 there were surprisingly already differences in odour profiles between eggs containing male and female embryos and this was still on day 8. (see paper for the compounds).

## **Conclusions/Discussion**

Both the fertility of an egg and the day of incubation affect the composition of







the odours of an egg during incubation. For fertile eggs the composition of the odours is predictive of the sex of the embryo, not only on day 8 but starting on day 1!!!!

## Fertile & infertile egg odour

The lack of odour difference on day 1 of fertile and infertile eggs was not surprising because embroynal development has just begun. On day 8, there is a significant difference in odour composition, presumebly due to a combination of the metabolism of the embryo and its use of the egg components which were not present in infertile eggs, including biochemical and microbial changes during development.

Birds invest a lot of time and energy in the production and incubation of eggs, and when the parent is able to detect the fertile eggs, this can be of advantage for descisions like leaving the nest or stay on it, with all the risks of being killed while brooding, the problems of finding food etc. So the smell of the eggs helps the parent to leave or stay and continue investing.

## Sex difference of embryos and egg odour

It was a huge surprise to find that even eggs which were brooded only 1 day, already showed a difference in smell between male or female embryos!!! It was expected NOT to find differences in this early stage since the embryos are hardly developed. It was expected to find a difference in odour at day 8 because at that time the embryonic growth rate or selective use of egg components could contribute to a difference.

Were it the embryos themselves on day 1 which contributed to the difference of odours or was it caused by the compounds the mother has given to eggs for manipulating male and female embryos?

This thought is not illogical since it is known female birds assign various yolk components to eggs in a sex specific manner (hormones, antibodies, vitamins, antioxidants), but it has never been researched whether these compounds change the smell of the eggs. Regardless the reason, it is proved that male and female embryos make an egg smell different. It may provide to give the parent a choice to selectively treat the individual eggs regarding sex of the embryo, maybe even adjust the growth rate depending on sex to enhance synchronous hatching. To make it more breaking: to give the parent the possibility to manipulate the sex ratio of the clutch! There, she has done it!!!

The above findings are beneficial for poultry industry as I wrote in the introduction, to detect the sex in earliest stage of development, avoiding post-hatch cruelties for male chicks in layer breeds... AND for us, just imagine... the egg-sex-sniffer, next to the incubator, so you can determine how many pullets and cockerels you want to breed this year, awwww!

Next to an increase of smelling compounds between day 1 and 8, there was also a decrease of some.

From chicken eggs it is known they can absorb odours from the environment and it is possible that some smells have been absorbed throught the porous shell during and after laying and are re-emitted at decreasing rates over the incubation period. If eggs are capable to absorb odours from outside (parent, nest) this might help recognising own egges although from Japanese quail it is known the recognise own eggs by pattern too, which in some cases will fail. A smell helps in such cases and chickens don't have patterned eggs so....

This study gives rise to further investigations like the communication between parent to embryo and interaction of embryos amongst each other, studies on development specifics long before they are visible in other ways like stress and prediction of infections and other things. It is also a start to find a commercially interesting system for very early sexing of production birds which would be a huge step forward in ethics and to us if we have the Egg-Sex-Sniffer!





Paper: Webster B, Hayes W, Pike TW (2015) Avian Egg Odour Encodes Information on Embryo Sex, Fertility and Development. PLoS ONE 10(1): e0116345. doi:10.1371/journal.pone.0116345, published: January 28, 2015

