The Pigeon Genetics Newsletter, News, Views & Comments.

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(Founded by Dr. Willard .F. Hollander)

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"T'is The Season To Be Jolly!!" Now we may not think that there is a Great Deal to be Jolly about , but look at your neighbor, or neighboring Country . There is always someone else who is having a rougher time of it no matter how bad things seem to be for you. That is why we are told to always count our Blessings!!

Elsie Lillian Cole Rodgers

Photo: My Mother 102 , her last Christmas with me 2014 - My Greatest Blessing for 71 years !

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You may have heard about or seen the University of Texas Report stating that they believe that smoky factor and Dirty factor are alleles , (both mutations at the same locus). I disagreed with this theory from the start and now we think we have good reason to say that these traits are not alleles. Jith has written a Review to this effect which we hereby present to you ---
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REVIEW of the Uof Texas Report on the allelism of smoky factor (sy) and Dirty factor (V). by Jith Peter , Kerala India.

Quite recently I happened to read the report on the allelism of dirty and smoky published by the University of Texas at Arlington. Among pigeon breeders it is believed that the mutations are at different locus, however according the report published by the university, they believe both mutations are at the Mc1r locus. They used the reverse genetics approach, wherein a genetic variation identified was then tried to be associated with the phenotypic variation it causes, to associate a large 500 base pair deletion mutation at the Mc1r gene with smoky phenotype, whereas in the normal forward molecular genetics approach, the genetic variation responsible for a phenotype will be determined. It is clear from the report that, the colour experts they brought in were able to identify smoky birds from the phenotypic variations. After identifying the smoky mutation, they set out to identify the genetic variation responsible for dirty. According to what they have said in the report, dirty was also segregating in the two families of birds, that they used to map smoky, exhibiting a peculiar inheritance pattern in which every individual expressed either smoky or dirty. **Though it was not clear from the report if they were able to see the phenotypic variation that is typical of dirty(V) and dirty-smoky on the birds tested, the latter if dirty and smoky are not alleles. They seem to have totally dismissed the option of both mutation expressing on the same bird, but it has been reported by many breeders and I myself have bred birds that seemed to have both mutations expressed, which, of course, seems to be a highly unlikely scenario unless they are not alleles, given that smoky and dirty are respectively recessive and dominant to their wild type counterpart.** They coupled the findings on smoky with breeding tests to determine that Dirty is allele to smoky and identified two independent dirty alleles, one of which is associated with melanic morphs in two other bird species. Substantial evidence which shows that smoky is an allele at the Mc1r locus is given in the report. However, the method they adopted to find out genetic variation causing dirty is rather precarious and not well substantiated.

What they did

The team found out a previously unknown 500 base pair deletion mutation at the Mc1r locus. Although many studies had been conducted on pigeon Mc1r gene in the past and many substitution mutations (Val85Met, Asp115Asn, Ser174Gly, and Arg217Cys) were found to be present at the locus, this deletion mutation was not reported previously. This was a surprising development considering the multiple previous studies (with inconclusive or conflicting results) of pigeon Mc1r coding variation. They then genotyped 6 birds from their laboratory breeding colony and found that 3 of them were heterozygous for the deletion mutation, including both members of a mated pair. Normal Mendelian segregation of the mutation was confirmed when they tested the offspring produced by the pair. Further molecular studies revealed the deletion mutation version is a null allele (loss-of-function mutation). They then attempted the reverse genetic approach to associate the genetic variation with possible phenotypic effects. **Similar loss-of-function mutations produce recessive red or yellow coats in mammals and so expected to have the**

same effect on plumage. Recessive red in pigeons would have been an ideal candidate for the deletion mutation, but we know from a previous study by the U of U that it is caused by a mutation at the enhancer of Sox10 gene. Since there was no apparent red pigmentation in the heterozygotes they genotyped, other major reddening traits, including kite, Archangel bronze 1, and Mahogany were excluded. In the absence of obvious large effect candidate traits, they adopted a family-based approach to limit the influence of epistatic factors and maximize sensitivity for traits of modest effect. Screening the founding pairs of pedigreed families from their laboratory colony identified two families segregating the deletion mutation. Family-1 a 14-member sibship from heterozygous parents and family-2 a large three generation filial pedigree derived from founders homozygous for alternative alleles **(here they did not mention if the founder parent without the deletion mutation was dirty or not, but the same bird would be mentioned as V//V later in the report).** Both families were noted to segregate for smoky.

Family 1: Parents – heterozygous smoky, total offspring 14, three of them were smoky.

Family 2: Parents – Sire: smoky and dam: non-smoky.

First generation(F1) offspring – 8 smoky carriers

Second generation(F2): F1s were mated inter se to produce 42 F2s, including 6 smoky individuals.

Mc1r genotypes of the founders of both families were consistent with (sy) locus genotypes. Independent phenotype assessments for smoky were made blind to genotype for all 65(or 64?) decedents of these founders by the color experts they brought. Phenotype calls were fully concordant between observers, and genotypes exhibited complete cosegregation between the deletion mutation and smoky: all smoky individuals were homozygous for the deletion, while all other individuals had at least one copy of the full-length allele, and obligate carriers were heterozygous for the deletion mutation.

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Based on the observation of both dirty and smoky segregating in the two families of birds exhibiting a peculiar inheritance pattern in which every individual expressed either dirty or smoky, they rejected the option of sufficiently small probability of such an inheritance pattern resulting from random segregation of unlinked loci and focused on the other two options.

1) Model 1 - Both families are fixed for dirty(V) and epistasis of homozygous smoky(sy) over dirty prevents the expression of dirty in smoky birds (that is a two-locus model with epistasis)
2) Model 2 - Dirty(V) and Smoky(sy) are alternative alleles at the same locus (or tightly linked)

The converse of model 1 was also an option but that was excluded because of the independent support for the association of 500 base pair deletion mutation at Mc1r with smoky seen in feral samples and the breed distribution data.

To discriminate between the model 1 and model 2 they used two F2 smoky birds from family 2 and mated them with unrelated non-dirty birds that were (by happenstance) heterozygous smoky.

Under the two-locus model, all offspring from such matings (sy/sy; V/V × Sy+ /sy; v + /v +) will either be smoky or Dirty, but never wild-type. Alternatively, if the allelism model is correct, these smoky F2 cannot harbor V, and all offspring of these sy/sy × Sy+ /sy matings will be either smoky or wild-type, but never Dirty. These test crosses produced a total of three smoky and six wild-type offspring, and no Dirty, establishing (sy) and (V) as alternative alleles of the same locus. They sequenced the family 1 birds and found the founder parents were both heterozygous for Gly174 variant and the V//V founder of family 2 was homozygous for the Met85 allele observed in the previous studies.

But imagine smokey and dirty are neither alleles nor linked as we breeders believe and then what if the smoky birds that they used for testcrosses lacked dirty factor or heterozygous dirty? The same breeding result is possible in either case. If the birds (F2s from family 2) indeed expressed dirty on the phenotype, then they couldn't have lacked dirty but could still be heterozygous dirty. The presence of Gly174 variant in the founder parents from family-1 and Met85 allele in the non-smoky founder parent from family-2 is a positive sign given the fact that one of the variants causes dark morphs in two other bird species. But the few numbers of samples they analyzed for this association can't be taken as a substantial evidence. They would have to test many dirty and non-dirty birds and see if all or a substantial number of them posses the variations at the Mc1r locus in the case of the former and the birds are free of these implicated alleles in the case of the latter.

They have mentioned in the report that, evaluations were supported by observations recorded shortly after hatching. But based on the phenotypic effects that were observed, all the birds they used were either smoky or wild type or dirty. They haven't mentioned at all the case of both dirty and smoky expressing on a single bird. It is of course not possible (under allelic model) if smoky is recessive to wild type and dirty is dominant to the other two. But birds expressing both dirty and smoky are rather common. Take for example beak color of adult birds, wild type and dirty birds show dark beak, smoky birds usually exhibit light beak with sometimes a dark spot at the tip and there is one more category; birds with horn colored beak, they were/are believed to be both dirty and smoky under non-allelic model. But how would you explain that under the allelic model? If smoky, a null allele, is recessive to the wild type with no signs of being carried in the heterozygotes, and dirtys, two substitution mutations according to the report, is dominant to the wild type.

The beak color may change during the first 2 to 3 months after the hatch and to see horn colored beak for a dirty smoky bird, one may have to wait till the bird reaches 2 to 3 months old, at least that is what happened with one of my smoky birds that I am going to present here.

Dirty is mostly unmentioned in the first part of the report where they explain how smoky was mapped. For instance, the non-smoky founder of family 2 was a homozygous dirty which they reveal later in the report when they explain how the substitution mutations were mapped. And the F1 offspring in the family 2 are mentioned as obligate heterozygotes. It is my impression that this was a deliberate choice with the good intention to not confuse the readers with both mutations at a time, especially because the research report implicates a paradigm shift from two mutations that have been considered neither linked nor alleles for decades to two (or three) alleles. However, I am afraid to say, if that was indeed the reason for dirty not being mentioned in the first part, the reason for the approach, since it is not made explicit in the report, can

possibly escape some readers, especially those who fail to see the big picture, and in them it can be a source of confusion.

Below is a part directly copied from the report that was given as "breeder lore"

"Breeders commonly credit the combined effects of smoky and Dirty for the quality of an individual solid colored pigeon, a proposition seemingly incompatible with sy being both allelic and recessive to V. Such claims appear to be founded on conjecture divined from the traits' separate effects in solid-colored backgrounds, rather than on rigorous genetic dissection; however, some may be more-or-less valid and yet still reconciled with our findings in either of two ways: First, breeders' selection for intense coloration by design accumulates minor uncharacterized darkening traits, and may do so in a manner cumulatively indistinguishable from V, thus permitting concurrent homozygosity for sy."

Of course, it is possible that there are more than one trait (non-allelic) that produces dirty phenotypic effects in pigeons. But, how could one say that the substitution mutations (if they indeed cause dirty phenotype, which is not conclusive from the report), happened at the Mc1r locus, and these mutations are the real dirty and the others are (i e, if there are) combined effects of more than one minor uncharacterized darkening traits? There may be other mutation/s that are able to produce as strong a phenotypic effect independently as the implicated dirty alleles at the Mc1r locus do. In such a case preferring to call one of them dirty over the other, just because that suits our purpose, would be misleading.

Below are Lalband-Ghagra, an Indian breed, bred by me for the Saffron project 4 to 5 years ago, all of them were bred in individual breeding pen. The parents were brought as adult birds. Dirty is common in the breed.

Male – Ghagra (Intense) Female 1 – Lalband(Intense) Female 2 – Zaraband(Dilute) Birds produced from the Ghagra cock and Lalband hen are shown in the photos that were taken when the birds were 4 to 7 days old.

1 st clutch, female and male

2 nd clutch, female and male.

4th Clutch, both females

5th Clutch, Male

1st Clutch, Male and Female

Light beak and feet were quite noticeable on one of the offspring in the 4th clutch from the Ghagra cock and Lalband hen and I waited to see how it would turn out. It turned out to be smoky exactly as I thought it would.

Females from 4th clutch after weaned

Females from 4th clutch after the first molt

Non-smoky female from 4th clutch

Smoky female from 4th clutch

Close profile view of head of the birds as adults from the 4th clutch - left non-smoky and right smoky.

Make no mistake, the breeding was done in an individual breeding pen and all the records and photos shown are accurate as it was done as part of finding a mutation responsible for the unique phenotypes present in the breed. If you are not familiar with Lalband- Ghagra, the breed is sexually dimorphic. Males are like ashred, but somewhat bluish all over. Females are rather like blue bars but somewhat different in colour tone all over. Both males and

females do have reddish orange pattern. There are some other colour variations present in the breed, but we don't need to go into detail with that now. The mutation responsible for the sexually dimorphic coloration was identified and named Saffron, a sex-linked, partial dominant mutation.

We bought the parent birds as adult, thus there was no observation made when they were in the nest. However, the colour of the offspring produced indicate the parents were dirty. The Ghagra cock, like the sons it produced with the two hens, had ashy tail and so it was impossible to say if it had albescent strips or not as the strips can't be seen on ashy tail (for instance, of ash-reds) unless some additional modifiers which darken the tail feathers are present. The female parents both had albescent strips. Both the intense parents had dark beak. All the offspring had dark skin, feet and beak as babies except for the smoky female, whose beak and feet were light as baby. All the offspring had dark beak except the smoky one, which showed horn colored beak as you can see from the photo. All the female offspring had albescent strips as adults, except the smoky one.

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Below is another observation made in a wild feral family which I used to keep. I come from the southern part of India where wild pigeons are mostly dirty.

Left two are parents and the rest offspring.

Two of the offspring as babies

The same birds after weaned

The same birds – one of them lacked albescent strips and the other one showed it partially. Both had similar skin colour as babies. However, the one lacked albescent strip had feet a bit lighter than the other one as baby and it was the one to first lose the melanin pigmentation from the feet.

As you can see neither of these breeding result support allelism of smoky and dirty.

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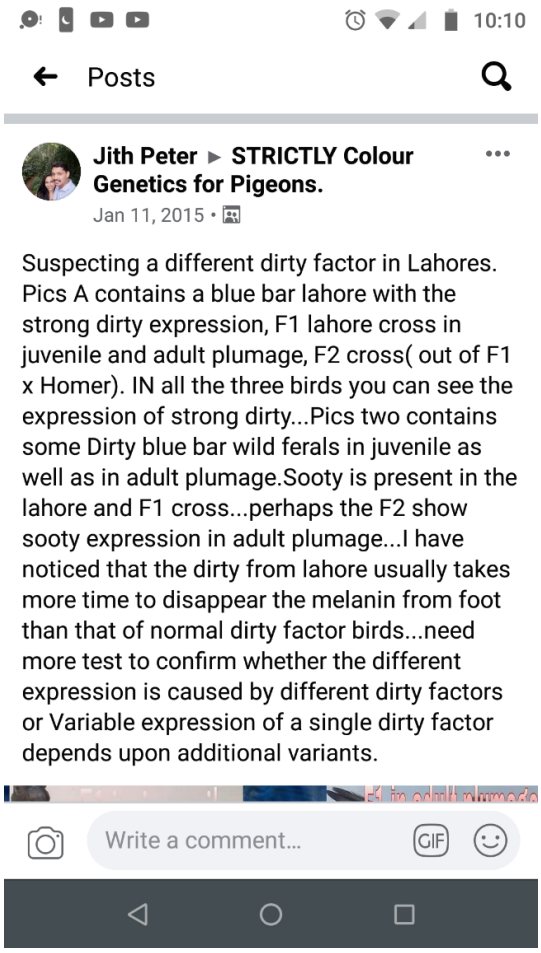
Below is a post I made on fakebook some years ago.



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That was a Review by co-Editor Jith Peter, of what was Reported by the University at Arlington Texas. We hope it offers some interest for your consideration. The subject is bound to draw a variety of opinions over the coming years. Hopefully that will bring about a definite conclusion .

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Here is a photo posted in the Facebook Group "100% Pigeons" by **Impreza Suburu Wrx**. Note that the youngster is clearly a smoky Blue checker lacking dirty. Both parents would have to carry smoky in order for this youngster to be produced , yet the blue checker parent demonstrates absolutely no hint that it carries the trait. One would also expect at least hetero Dirty to be expressed in the young if indeed that trait was in either parent as it appears to be in the black.

Topic #2.. Black agates ??

One question that keeps popping up is the idea that in the old days there seems to have been a Black pigeon with a mottled or rosewing white marking combined with a few white feathers also speckled over the heart shape area of the back of the pigeons. It is commonly called a 'handkerchief' marking. It was rather common in writings about the Classical Almond and therefore thought to imply that it could be part of the genome of Classical Almonds particularly the English Short Face Tumbler Breed. **It was sometimes referred to as a black Agate., which of course it was not**.

We have to keep a number of things in mind here. (1) The phenotype suggests one of two genetic traits, either Tiger grizzle or Print Grizzle. (2) The Black base suggests SPREAD factor, a modifier not used in Classical Almond Breeding nowadays. (3) Back in those days of early writing they had no idea what Spread factor was and therefore no idea how it would or would not work when combined with other traits.

They combined the mutation 'Stipple' with various basic traits and the combination with spread factor produced the Black Sprinkle. Ironically a homozygous Tiger grizzle can make a very good look-alike for a Sprinkle. It seems likely therefore, that grizzles were indeed part of many early Almond breeding programs and no distinction between actual stipple sprinkle and homo Tiger or hetero Print spread Grizzle were realized. The term 'Ermine' was used for these black & white 'sprinkles'.

Print Grizzle plus spread factor also gives a range of 'mottled' phenotypes , and very possibly would produce Sprinkle-like phenotypes still not realized to this day. Heterozygous Tigers and Prints often are easily confused by breeders even today .

The New Year will offer us an opportunity to dwell on each of the Topics that , like Black Agate, need to be examined and have many of the myths dispelled !

We will bring photos that will demonstrate what we are referring to in each case .

That is it for December and the Year 2020 ! We sincerely hope it will be the restart of getting back to normal for everyone around the World. This year has been a wakeup call for all of us. We know that many aspects of our lives need to change for the better yet we are a species that hates change! Now is the time to seriously consider YOUR New Year's Resolution , and stick to it !

That is it from the Loft for now , See all of you in January if HE sees fit !