Plumage colour variations in the *Agapornis* genus: A review

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ABSTRACT

The genus *Agapornis* consists of nine small African parrot species that are globally well known as pets, but are also found in their native habitat. Illegal trappings, poaching and habitat destruction are the main threats these birds face in the wild. In aviculture, *Agapornis* breeding is highly popular all across the globe. Birds are mainly selected based on their plumage colour variations but very little molecular research has been conducted on this topic. There are 30 known colour variations amongst the nine species and most of these are inherited as Mendelian traits. However, to date none of the genes or polymorphisms linked to these variations have been identified or verified. Due to unethical breeding practices the need for the development of molecular tests such as identification verification tests or species identity tests is growing. Future research is paramount to ensure the conservation of wild populations as well as aiding breeders in improving breeding strategies.

Keywords: Psittacidae, lovebirds, aviculture, molecular breeding tools

INTRODUCTION

Globally, there are 356 extant psittaciform, or parrot, species (Forshaw, 1989) of which twenty are native to the African continent and Madagascar (Perrin, 2009). Amongst this order, the genus *Agapornis* (Selby, 1836) is found and consists of nine species, namely *A. roseicollis*, *A. taranta*, *A. canus*, *A. personatus*, *A. lilianae*, *A. swindernianus*, *A. fischeri*, *A. pullarius* and *A. nigrigenis* (Moreau, 1948; Dilger, 1960; Forshaw, 1989). These species are more commonly known as lovebirds and are distributed across Madagascar (*A. canus*) and Africa (the remaining eight species) (Dilger, 1960; Forshaw, 1989; Perrin, 2012). Table 1 lists the distribution of each species, size of the birds, known sub-species, common name and popularity of each species in aviculture. In addition to existing in their natural habitat, lovebirds are popular pets and eight of the nine species, *A. swindernianus* being the exception, are kept and bred as domestic birds (Hayward, 1979; Silva & Kotlar, 1989; Forshaw, 1989; Van den Abeele, 2016).

Lovebirds have been kept as pets since the eighteenth century with reports of *A. pullarius* being bred in Britain in the 1880's (Parkes, 1982). Hayward (1979) states that lovebirds are popular in aviculture due to the fact that they breed relatively easy in captivity, are hardy, active and have a range of plumage colours. They can also be bred in a small aviary making them ideal birds for today's modern lifestyle. As a consequence of their popularity in aviculture, birds in their natural habitat are being threatened by poaching and trapping for export to pet markets (Warburton & Perrin, 2005 & 2006; Mzumara *et al.*, 2016 a & b). Plumage colour variations are the main economic factor breeders select for and there are at least 30 colour variations amongst domestic lovebirds that are accepted by shows and auctions (Hayward, 1978, Van den Abeele, 2016). Rare colour variants are sold for up to 700 times as much as a wild type coloration bird of the same species in Europe (Personal communication: Mr. Dirk Van den Abeele) and there are reports of mark-ups of up to 1300 times in Asia (Diega, 2017). All of these colour variants are genetically inherited, but to date the only reports of the mode of inheritance of these traits are breeders' records. Due to many of these colour variants being recessively inherited, it may lead to inbreeding of related birds in order to create a recessive plumage genotype. Since some lovebird species can inter-mate, hybridization of different species is also of great concern in aviculture.

Despite their popularity, very little molecular genetic research has been conducted on these species, in particular for plumage coloration inheritance. Currently the genes and polymorphisms linked to lovebird coloration are not yet known and therefore breeders cannot screen young birds to confirm their colour genotype. There is also no screening test to identify species nor a species-specific parentage panel or identity confirmation test available. These tests could be useful not only in aviculture but also in conservation to monitor natural populations.

This paper reviews the importance of the conservation of the lovebird species in their natural habitat as well as the role these birds play in aviculture. The mechanisms of parrot plumage colour expression will be discussed as well as the different colour variations that are found in this genus and the importance thereof as an economic driving factor for these species. The lack of genetic screening tests makes genetic progress in aviculture difficult and this paper briefly investigates the different diagnostic tests that could improve lovebird breeding.

GENUS *AGAPORNIS*

Lovebirds have been domesticated since the 1880's but surprisingly little molecular genetic research has been conducted on these species. The first taxonomic classification of the species was done by Moreau (1948). The

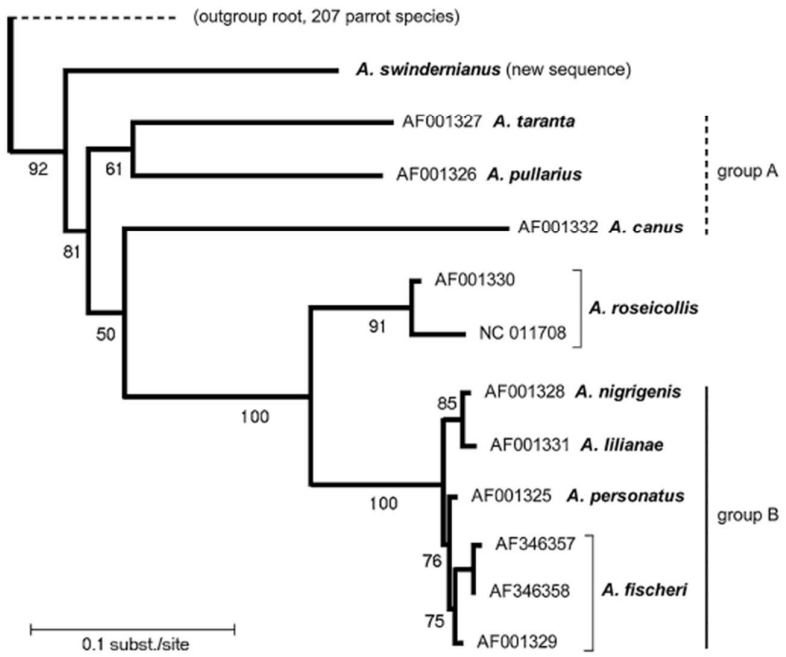

Figure 1. The classification of the nine *Agapornis* species as made by Manegold & Podsiadlowski (2014) (Adapted from Manegold & Podsiadlowski, 2014).

nine extant species were classified into two groups (Group A and B) with two sister taxon groups consisting of *A. roseicollis* and *A. swindernianus* respectively, as can be seen in Figure 1. This classification was revised by Dilger (1960) who classified them only as three different groups based on the presence or absence of the white eye ring, whether they are monomorphic or not and an intermediate group.

Molecular genetic advances gave researchers the opportunity to study these birds on a more detailed genetic level in the late 1990's. Eberhard (1998) sequenced a portion of the cytochrome-b (cytb) mitochondrial gene of eight *Agapornis* species (with the exception of *A. swindernianus*). The molecular evidence from this study confirmed the classification by Dilger (1960) and categorized the species as the eye-ring group, sexually dimorph group and transitional group. In a later study by Manegold & Podsiadlowski (2014) the cytb mitochondrial gene of a museum sample from *A. swindernianus* was additionally analysed and *A. swindernianus* was found to be a sister clade to all other *Agapornis* species (Figure 1). *A. canus*, *A. pullarius*, *A. swindernianus* and A. *roseicollis* all have known subspecies (Table 1). Some of the sub-species are poorly differentiated and researched (Forshaw, 1989; Van den Abeele 2016).

Karyotyping of the *A. roseicollis* genome by Nanda *et al*. (2007) revealed that this species has a diploid number of 48 macro-chromosomes. In the same study the budgerigar (*Melopsittacus undulates*) was found to have 2n = 62 macro-chromosomes. The lower number of *Agapornis* chromosomes might indicate a fusion of ancestral micro-chromosomes (Nanda e*t al.*, 2007). The budgerigar had a marginally larger genome size of 1.2 Gb (Ganapathy *et al.*, 2013) compared to the *A. roseicollis* genome of 1.1 Gb (van der Zwan *et al.*, 2018).

Crossbreeding between some of the species is possible and can produce viable hybrids (McCarthy, 2006; Van den Abeele, 2016). The species' habitat ranges do not overlap and therefore interbreeding is rarely found in the wild. The only habitat overlap is between *A. personatus* and *A. swindernianus* (Moreau, 1948; Dilger, 1960; Forshaw, 1989) but these species' behaviour is so different that, even though not well researched, it is doubted that interbreeding occurs. In aviculture, unfortunately, it is not uncommon to see hybridization of species. Hybrids are not generally accepted at auctions and shows (Van den Abeele, 2016) and crosses between species should not be encouraged in aviculture. McCarthy (2006) list the possible observed crosses between *Agapornis* species that could produce viable young and a summary is given in Table 2 (viable young born depicted with an "x").

Viable offspring hatching from a mating between an *A. canus* male and *Melopsittacus undulatus* (Budgerigar) female, *A. nigrigenis* male and *M. undulates* female, *A. personatus* male and *M. undulates* female and *A. roseicollis* male and *M. undulates* female (McCarthy, 2006), have also been reported. It is well known that some

Table 1. A summary of *Agapornis* species

Species	Distribution	Size	Sub-species	Common name	Aviculture	References
A. canus	Madagascar	14cm	*A. c. canus* and *A. c. ablectaneus*	Lavender head, grey head, Madagascar lovebird	Less common	Forshaw, 1989; Haywards, 1979; Van den Abeele, 2016
A. taranta	Ethiopia, Eritrea and Djibouti	17cm	None	Black-winged or Abyssinian lovebird	Less common	Forshaw, 1989; Marez, 2003; Tekalign & Bekele 2011, Van den Abeele, 2016
A. pullarius	Across the African equator from Kenya to Guinea over 26 countries	15cm	*A. p. pullarius* and *A. p. ugandae*	Red-headed lovebird	Uncommon	Silvia & Kotlar, 1988; Forshaw, 1989; Van den Abeele, 2016; Egwumah & Iboyi, 2017
A. roseicollis	Angola, Namibia, Botswana and South Africa	16cm	*A. r. roseicollis* and *A. r catumbella*	Peach-faced or rosy-faced lovebird	Very common	Forshaw, 1989; Ndithia *et al.*, 2007; Van den Abeele, 2016
A. swindernianus	Cameroon, Gabon, Congo, CAR, DRC and Uganda	13cm	*A. s swinderninus* *A. s zenkeri* *A. s emini*	Black-collared lovebird	Not found	Forshaw, 1989; Manegold & Podsiadlowski, 2013

Species	Distribution	Size	Sub-species	Common name	Aviculture	References
A. personatus	Tanzania	15cm	None	Masked or black-masked lovebird	Very common	Forshaw, 1989; Perrin 2009, Van den Abeele, 2016
A. lilianae	Zimbabwe, Zambia and Malawi	14.5cm	None	Lilian's lovebird	Very common	Forshaw, 1989; Mzumara *et al.*, 2016 a & b; Van den Abeele, 2016
A. fischeri	Tanzania	15cm	None	Fischer's lovebird	Very common	Forshaw, 1989; Mwangomo *et al.* 2007; Van den Abeele, 2016
A. nigrigenis	Zambia	14cm	None	Black cheeked lovebird	Very common	Forshaw, 1989; Warburton & Perrin, 2005 & 2006; Van den Abeele, 2016

Table 2. Possible viable matings amongst domestic *Agapornis* species.

	A. canus	*A. fischeri*	*A. nigrigenis*	*A. lilianae*	*A. personatus*	*A. roseicollis*	*A. pullarius*	*A. taranta*
A. canus		x	x	x	x	x	x	x
A. fischeri	x		x	x	x	x		x
A. nigrigenis	x	x		x	x	x		
A. lilianae	x	x	x		x	x		
A. personatus	x	x	x	x		x	x	x
A. roseicollis	x	x	x	x	x		x	
A. pullarius	x				x	x		x
A. taranta	x	x			x			

plumage colour variations were introduced to other *Agapornis* species by intentional crossing of the species that can intermate (van den Abeele, 2016).

Ecology and threats to natural populations

The behaviour and habitat of the nine species varies greatly - *A. swindernianus* are exclusive forest canopy dwellers, *A. lilianae* prefer river beds and Mopane woodlands, *A. taranta* lives at high altitudes of 1600 to 3800 meters, *A. pullarius* are lowland tropical birds, *A. roseicollis* is found in semi-arid environments, *A. fischeri* and *A. personatus* are lowland savanna and wood-land dwellers, *A. canus* prefers lightly wooded habitats and *A. nigrigenis* can be found in mopane (*Colophospermum mopane*) woodlands (Foshaw, 1989; Perrin, 2009; Van den Abeele, 2016). The main diet of most of the *Agapornis* species consist of grass seeds, however *A. swindernianus* feed on ripened figs (Forshaw, 1989; Perrin, 2009). Moreau (1948) notes that *A. swindernianus* cannot survive in captivity if not fed figs, which could explain why these birds are not kept as pets.

The International Union of Conservation of Nature (IUCN) lists *Agapornis nigrigenis* as vulnerable, *A. fischeri* and *A. lilianae* as near threatened and the other species as least concern. All species except for *A. roseicollis* are classified by The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) as CITES Appendix II species, indicating that they are not threatened with extinction, but trade must be controlled to prevent their extinction. Several studies have been conducted on the natural lovebird populations and the threats these birds are faced with. In conclusion Tekalign & Bekele (2011) (on *A. taranta*), Mzumara *et al.* (2016 a & b) (on *A. lilianae*), Egwumah & Iboyi, 2017 (on *A. pullarius*) and Perrin (2012) (all species) found that reduction of their natural habitat due to agricultural activity and trapping or poaching is the main threats to these species. In addition, Mzumara *et al*., (2016a) also found that the poisoning of birds in Malawi is a common practice as these birds destroy crops.

Worldwide, there is a keen interest in lovebird breeding. Membership of the world's largest breeding association - The Belgium Agapornis Breeders Association (BVA-International) - is approximately 5000 members (Personal communication, Mr Dirk Van den Abeele) and other societies across Europe, Africa, Australia, the United States of America, South America and Asia (Van den Abeele, 2016) exists with various member numbers. In a recent poll conducted by Ornitho Genetics VZW (2017), the most popular lovebird species bred in captivity was *A. fischeri,* followed by *A. personatus* and *A. roseicollis*. The popularity of a species in aviculture could drastically influence their numbers in the natural habitat as traders will trap these birds in order to sell them. Perrin (2012) reports that *A. roseicollis*, *personatus* and *fischeri* are all heavily traded

whereas *A. lilianae* are traded moderately and *A. nigrigenis* to a lesser extent. Trade and trappings are banned in several African countries which should have an effect on the natural populations' growth.

Aviculturists have the responsibility to breed with purebred, healthy birds to ensure that if the wild populations go extinct, these species will still survive in aviculture. With the use of molecular diagnostic tests, breeders will be able to select birds with recessive colour variations without compromising the biodiversity of the species. Unrelated birds that are heterozygous for a recessive colour trait, could be identified and mated, rather than mating close relatives that are suspected heterozygotes. Breeders should be made aware of the importance of conserving wild populations as well as ethical breeding practices. This could be attained by promoting the importance of not selecting breeding pairs based on one trait (colour) only, but to keep biodiversity and general genetic fitness in mind.

AGAPORNIS PLUMAGE COLORATION

As mentioned earlier, the main economic factor breeders select for is plumage coloration and many of these are inherited as autosomal recessive traits (Table 3). This implies that an offspring with wildtype coloration has a certain chance (depending on the parents' genotypes) of being a heterozygote (thus a carrier or split) of this trait. Breeders sell possible heterozygotes at a premium despite the fact that the buyer has neither proof that the bird is an offspring of the colour variant nor that it is indeed a heterozygote for the colour variation. This could lead to fraudulent transactions and emphasises the need for more research in the field of plumage colour genetics and parentage verification testing for lovebirds.

Agapornis plumage colour and biological mechanisms controlling parrot coloration

Most of the Agapornis species has at least one colour variation that is found infrequently in wild populations and, due to selective breeding, frequently in domestic populations. The main colour of all wild type *Agapornis* birds is green with red, yellow, pink, black or grey on their heads, chest and neck and some blue in their tail feathers, (Hayward, 1978, Silva & Kotlar, 1988, Forshaw, 1989; Van den Abeele, 2016), as seen in the photos of the wildtype coloration of the eight domestic *Agapornis* species in Figure 2. In Figures 3 a- h some of the colour variants of the different species are shown with the wildtype variant of that species. Reports on wild lovebirds with colours other than the wildtype coloration is scarce but Warburton & Perrin (2005) observed one yellow and three light-green *A. nigrigenis* birds in the mid Machile River region between May 1999 and May 2000. Van Den Abeele (2016) identified a wild-caught double factor green *A. roseicollis* in Namibia and in the

Table 3. Plumage colour variations and their inheritance patterns found amongst *Agapornis* species

	A. roseicollis	*A. canus*	*A. taranta*	*A. pullarius*	*A. personatus*	*A. fischeri*	*A. lilianae*	*A. nigrigenis*	Inheritance pattern
Dark factor	X		X		X	X	X		AID
Blue					X	X	X	X	AR
Aqua	X					X			AR
Turquoise	X		X						AR
Orange face	X								AR
Pale headed	X								AID
Opaline	X					X			SLR
Ino (NSL[#])					X	X	X	X	AR
Ino (SL[†])	X								SLR
Pastel					X				AR
Dark eyed clear						X			AR
Pallid	X								SLR
Cinnamon	X								SLR
Pale	X					X			SLR
Marbled	X								AR
Dilute	X		X				X	X	AR
Bronze fallow	X		X			X			AR
Pale fallow	X		X			X			AR
Dun fallow					X				AR


Figure 2 a


Figure 2 b


Figure 2 c


Figure 2 d


Figure 2 e


Figure 2 f


Figure 2 g


Figure 2 h

Figure 2. Eight domestic *Agapornis* species with wildtype coloration.
Figure 2a: *A. canus* (male left, female right).
Figure 2b: *A. taranta* (male left, female right).
Figure 2c: *A. pullarius* (male left, female right).
Figure 2d: *A. roseicollis*.
Figure 2e: A. *personatus*.
Figure 2f: *A. fischeri.*
Figure 2g: *A. nigrigenis*
Figure 2h: *A. lilianae*.
(All photos: Mr Dirk Van den Abeele).

Figure 3 a

Figure 3 b

Figure 3 c

Figure 3 d

Figure 3 e

Figure 3 f

Figure 3 g

Figure 3 h

Figure 3. Wildtype coloration and some colour variations of four *Agapornis* species
Figure 3a: *A. fischeri*: Wildtype
Figure 3b: *A. fischeri*: Turquoise
Figure 3c: *A. personatus*: Wildtype
Figure 3d: *A. personatus*: blue (blue left, DD blue middle – (homozygous dark factor), D blue – (heterozygous dark factor - right),
Figure 3e: *A. lilianae* Wildtype
Figure 3f: *A. lilianae Non-Sex linked ino green*
Figure 3g: *A. roseicollis*: Wildtype
Figure 3h: *A. roseicollis*: Opaline d blue
(All Photos: Mr. Dirk Van den Abeele).

1932 Proceedings of the Zoological Society of London a wild-caught *A. personatus* male was the first blue *A. personatus* documented. Some of these variations have been known to breeders for almost a century. Toerien (1950) reports a lutino *A. roseicollis* bred from a wild caught population from Namibia, whereas Delacour (1942) reports the same variation in *A. fischeri* and Moreau (1948) in *A. lilianae*.

In most avian species, plumage coloration is affected by genetically controlled dietary intake of carotenoids (Sage, 1962; Guay *et al*., 2012), but in the case of parrots, diet has little or no influence on coloration as it is mainly due to genetic variation (Hill & McGraw, 2006). Black, brown and red-brown plumage, beak, claw and eye colours in all bird species are caused by the pigment melanin, whereas red, yellow, pink and orange colours found in parrot plumage are caused by psittacofulvin pigments (Mundy, 2006; Hill & McGraw, 2006). Parrots are the only group of organisms known to synthesize psittacofulvin pigments in their plumage cells (Stradi *et al*., 2001; Hudon & Brush, 1990; McGraw & Nogare, 2005, Hill & McGraw, 2006). Pigments (melanin and psittacofulvins) and structural changes in the feather barbs are the two mechanisms that produce the various parrot colours (Stoddard & Prum, 2011). Physical interactions of light waves with β-keratin rods in the feather barbs produces purple, blue and green colours (Dyck, 1971 a & b; Prum *et al*., 1994; Prum, 2006). The two mechanism work together as follows: pigments (melanins and psittacofulvins) absorb and reflect light selectively (D'Alba *et al*., 2012) and due to the structural changes in the feather barbs the light is reflected irregularly (Dyck, 1971 a & b; Hill & McGraw, 2006; Berg & Bennett, 2010). The barb of a green feather, for example, contains a cortex filled with yellow psittacofulvin pigment. Light is partially absorbed by the eumelanin pigment in the feather, and blue light is reflected back through the air vacuoles. It then passes through the yellow psittacofulvin pigment and through coherent scattering of the quasi-ordered β-keratin rods, the light (and ultimately the feather) appears green (Dyck 1971 a & b). Dark green feathers reflect about half the light compared to lighter green feathers (Dyck, 1971 a & b). Mundy (2006) stresses the fact that these two mechanisms (pigment and structural changes) are most probably under independent genetic control but that no candidate genes have been associated with structural coloration control.

Agapornis plumage variations

Buckley (1987) defines a plumage colour polymorphism as the presence of a plumage colour aberration in an interbreeding population of individuals of the same sex and age. There are at least 30 known naturally occurring plumage colour variations amongst six of the domestic *Agapornis* species (Van den Abeele, 2016). The inheritance pattern of these variations have mainly been determined by test matings performed by breeders, and

very little scientific literature is available on this subject. MUTAVI, a parrot breeding research and advice group located in The Netherlands (www.MUTAVI.info) conducted research trials on the structures of *Agapornis* feathers of various colorations. In Table 3, a summary of the different plumage colour variations per species plus their inheritance patterns, is given. Given that none of the polymorphisms linked to these colour variations have been identified, the colour patterns will be referred to as "plumage colour variations" and not “plumage colour polymorphisms”.

There are four main categories of colour variations in *Agapornis* and a discussion with examples thereof follows below. These include variations due to psittacofulvin reduction or modification, variations due to a change in melanin, change in feather structure and changes in eumelanin. It is important to note that many of these plumage colour variations can be combined that will result in a range of plumage colours (Hayward, 1979; Van den Abeele, 2016). Berg & Bennett (2010) as well as Mundy (2018) highlights the lack of research on the genetic control of aberrant colours in caged parrots.

Aberrant plumage coloration due to psittacofulvin reduction or modification

Some of the most dramatic colour variations amongst the psittaciform order, is caused by the reduction or modification of psittacofulvin within the feather cortex. In the 1932 Proceedings of the Zoological Society of London a wild-caught *A. personatus* male was described as “the yellow coloring was absent, the areas of the body normally yellow, being whitish and those normally green, blue.” Cooke *et al*. (2017) reported that since the 19th century budgerigars bred in captivity has displayed a loss of psittacofulvin pigmentation causing birds to have blue, instead of the wildtype green, plumage coloration. Yellow pigment in the cortex of the feather is normally combined with blue light and results in visibly green feathers. Cooke *et al.* (2017) found that due to a T>C substitution mutation in the *MuPKS* gene in budgerigars, no yellow psittacofulvin pigment is produced and therefore blue light is reflected so that the feather appears blue. Other areas of the body that would normally be red, yellow or orange due to psittacofulvins are white due to the absence of the psittacofulvin pigment (Prum, 2006; Van den Abeele, 2016). The same phenotype is observed amongst lovebirds and it is also inherited as an autosomal recessive trait, as in budgerigars. Research done at MUTAVI (unpublished, personal communication Mr Dirk Van den Abeele) has found that no yellow psittacofulvin pigment was present in blue lovebirds compared to pigment being present in wildtype green birds. More research on the molecular basis of this gene in lovebirds is needed before conclusions can be made for this genus.

In contrast to the *Blue* phenotype where no psittacofulvin is produced, birds with *Aqua* and *Turquoise* phenotypes produce a reduced amount of psittacofulvin. There are no reports of studies where the actual pigment is measured, but visibly about 50% of the normal amount of psittacofulvin is produced and only about 40% of the normal levels of psittacofulvin is visible in birds with the *Turquoise* variation (Van den Abeele, 2016). It remains to be confirmed if a *MuPKS* polymorphism is causative to these phenotypes

Orange face and *Pale headed* are two popular phenotypes in lovebird aviculture and are only found amongst *A. roseicollis* (Van den Abeele, 2016). The mechanism behind these phenotypes are poorly understood (Personal communication, Mr Dirk Van den Abeele) but for *Orange face* individuals, red plumage is changed to orange, whereas in *pale headed* individuals the psittacofulvin change from red to pink. Green plumage remains unchanged for both phenotypes. Research by McGraw & Nogare (2005) indicated that the red, orange and pink coloration are all due to psittacofulvin pigment in different concentrations and therefore these colour variations could be caused by a change in psittacofulvin pigment concentration, but this remains to be confirmed.

Colour variations due to a change in melanin

Mundy (2006) and McGraw (2006) noted that most colour variations are caused due to changes in melanin distribution. However, little to no molecular research has been conducted on parrot species regarding the genetic basis of these variations. Most of the common types of bird plumage colour variations can be greatly attributed to a lack of melanin in the feathers. Examples of plumage coloration variations due to a change in melanin in *Agapornis* is briefly given in Table 4.

Colour variations due to a change in the feather structure

The "*dark factor*" genotype causes the spongy zone of the feather to decrease in size. Due to this reduction only about half the light is reflected throughout the visible spectrum compared to light green feathers (Dyck, 1971 a & b). The inheritance of this phenotype is incomplete dominance and three phenotypes are observed. Heterozygote birds (also called "*single dark factor*") are slightly darker (a khaki green) than the wildtype and birds that are homozygous for the mutation ("*double dark factor*") are found to be an olive green colour (Hayward, 1979; Van den Abeele, 2016; Unpublished data, MUTAVI). This phenotype is observed in five of the *Agapornis* species as seen in Table 3. It is also found amongst the budgerigar resulting in the same colours (Harris, 1979).

Research projects conducted at MUTAVI, discovered that lovebirds with the *slaty* phenotype had a change in the keratin in the feathers from a horn colour to a glassy see-through colour (Personal communication: Mr. Dirk Van den Abeele) and plumage colour change to a steel blue colour. A similar phenotype, called *slate*, is commonly found amongst budgerigars (Elliott, 2013) and is caused by changes in the feather structure and inherited as a sex-linked recessive trait. Through test matings it was determined that this phenotype is inherited as an incomplete dominant trait. However, there is visually no difference between a heterozygote and a homozygote for the mutation and therefore genotypes cannot be determined based on phenotype alone. (Van den Abeele, 2016). Further research is necessary to identify the gene and polymorphism linked to these phenotypes and confirmation whether the *slaty* and *slate* phenotypes are caused by the same polymorphism.

The *violet* phenotype develops due to a structural change in the spongy zone of the feather. It is however only visible when it is combined with the blue phenotype. If psittacofulvin is absent in the cortex (thus a blue bird), combined with the structural change in the spongy zone, the bird appears violet. The same phenotype is found in budgerigars with the same underlying inheritance pattern (Harris, 1979; Van den Abeele, 2016). There is a difference in appearance between a heterozygote and homozygote for the mutation in both of the species.

Coloration due to changes in eumelanin

In 2017 the "*Jade*" phenotype was officially accepted as a plumage colour aberration in *A. roseicollis*, and is inherited as an autosomal recessive trait. Interestingly the breeders of these birds observed that females are lighter than males. This phenotype has not been described in the literature and all observations are from personal communication with Mr. Dirk Van den Abeele. Birds with this variation appears to have a visible reduction of about half of the eumelanin in their wing feathers and more so over their body, resulting in a yellow colour with a darker hue. There is a greater eumelanin reduction in the core of the wing feathers which results in a slight edge on the feathers. The bird's rump is much lighter and the legs are of normal coloration, but the nails are darker. The mask of these birds are of normal coloration.

The *Euwing* pattern is a colour variation only found amongst A. *fischeri* causing darker wings and an overall different body colour. The cause of the phenotype is still unclear but is inherited as a dominant trait with incomplete penetrance (Van den Abeele, 2016). Veeckmans (2016) notes that the *euwing* mutation "dulls" the black eumelanin pigment in the body feathers and on the mantle in heterozygote birds. In homozygous affected birds, the effect is much more prominent with additional eumelanin found in the wing covert feathers, making

the bird appear darker. By combining a bird with both the *euwing* mutation as well as the *opaline* mutation, breeders can achieve spectacular colorations.

PARENTAGE VERIFICATION

Applying molecular techniques to confirm parentage has been well documented for various domesticated animal species including cattle (Matukumalli *et al.*, 2009); goats (Visser *et al*., 2011) and dogs (DeNise *et al.*, 2003). Marker panels for parentage verification in birds are also common, however these panels are applied more in conservation than breeding (Jan & Fumagalli, 2016; Coetzer *et al.*, 2017). Most of these studies used microsatellite markers but in recent years the focus has shifted towards the use of Single Nucleotide Polymorphisms (SNPs) (see Weinman *et al.* (2015) for a comparison). Identifying incorrect pedigrees are of importance in livestock populations as it adversely influences genetic gains (Israel & Weller, 2000). Although *Agapornis* species are not under directional selection for increased genetic gain, incorrect pedigrees will still negatively affect the populations' general fitness. Despite breeders' efforts to separate breeding pairs and recording the offspring, misidentification of parents still occurs. Due to the fact that many of the colour variations are inherited as autosomal recessive traits, breeders may deliberately sell a young as an offspring of a bird with the colour variation knowing there is no way of confirming either parentage or the colour genotype. This creates a serious loophole that could be exploited by unethical breeders. Currently there is no species or genus specific parentage and individual identification panel available for lovebirds. The development of such a panel could benefit breeders, buyers and conservationists in not only conserving these species but also ensuring ethical breeding practices.

SPECIES IDENTIFICATION

Rheindt & Edwards (2011) reports that hybridization, as well as the resulting genetic introgression, is not uncommon in birds. In aviculture the crossing of two species might result in the introduction of a novel colour variation in a different species. Since birds from some of the different *Agapornis* species can inter-mate (see Table 2), many of the variations were transferred through genetic introgression from one species to another. For example, the "*dark factor*" phenotype was first observed in *A. roseicollis* in 1968 and in the 1980s the first dark factor *A. personatus* was identified. From there the phenotype was transferred to *A. fischeri*, *A. lilianae* and *A. nigrigenis* by interbreeding of the species (Van den Abeele, 2016). Responsible aviculturists should take care not to interbreed species just to develop "new" colours in a species or to develop a "new species" of *Agapornis*.

Hybrids are not generally accepted by breeding societies, but unknowing buyers might purchase a hybrid and breed further with this bird, leading to an even larger hybrid population. In addition, conservationists and custom officials can benefit from a species identification test to confirm species from a tissue sample. Genetic testing to infer if a species or if an individual is a hybrid between two species has been applied frequently in aquaculture (Rasmussen & Morrissey, 2008; Hohenlohe *et al.*, 2011). Von Holdt *et al.* (2011) reports the application of SNPs to distinguish between dog and wolf hybrids and Koutsogiannouli *et al*. (2010) applied Restriction Fragment Length Polymorphisms (RFLPs) to identify hybrids between wild and domestic pigs. The development of a similar panel of SNPs could aid breeders, breeding societies, buyers, conservationists and custom officials in identifying from which species an individual or a tissue sample originated and whether the individual is a purebred or hybrid bird.

THE ROLE OF AVICULTURE IN CONSERVATION OF *AGAPORNIS* SPECIES

As with many other domestic parrot species, some of the wild *Agapornis* species are under pressure due to, amongst others, illegal trade to the pet market. The development of the genetic screening tests such as the identity verification and species identification tests could aid conservationists in preventing extinction of these species. Fortunately, none of the nine species are extinct yet but should the wild populations become extinct, captive breeding populations could be used in programs to reintroduce these species to the wild. This, however might not be a simple task due to genetic and phenotypic changes that occur in captivity (Snyders *et al.*, 1996). Domestication and tameness are additional problems since behaviour changes set in fairly quickly when a species is selected for tameness and these birds could struggle to adapt to a natural lifestyle, especially in a habitat with predators (Snyders *et al*., 1996). Conservationists and breeders should therefore combine their efforts to firstly ensure the survival of the wild populations and secondly breeding for show or pet market. This can be achieved by making breeders aware of the threat wild populations face and the importance of not only selecting for one trait (colour) but to keep genetic diversity, inbreeding level and species in mind when a mating is planned. Conservationists should continue monitoring wild population growth and should the need for a captive breeding program with the aim to release domesticated birds to the wild arise, utilise genetic screening tests to plan these programs.

CONCLUSION

The popularity of lovebirds in aviculture coupled with their reduced habitat and poaching has placed the natural populations of these birds under strain. It is the aviculturists' duty to breed the birds responsibly to ensure the survival of these species should the wild population become extinct. More research is needed in the field of avian genetics to determine the causative genes and mutations linked to colour variations and to develop genetic screening tests to determine the genotype, species and pedigree of the bird.

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REFERENCES

Berg, M.L. & Bennett, A.T.D. (2010) The evolution of plumage colouration in parrots: a review. *Emu*, 110, 10 – 20.

Buckley, P.A. (1987) Avian Genetics: A population and ecological approach. Academic press, Harcort Brace Jovanovich, Publishers.

Coetzer WG, Downs CT, Perrin MR, Willows-Munro S. (2017) Testing of microsatellite multiplexes for individual identification of Cape Parrots (*Poicephalus robustus*): paternity testing and monitoring trade *PeerJ* 5.

Cooke, T.F., Fischer, C.R., Wu, P., Khosla, C., Chuong, C-M., Bustamante, C.D. (2017) Genetic mapping and biochemical basis of yellow feather pigmentation in budgerigars. *Cell*. 171, 427 – 439.

D'Alba, L., Kieffer, L., Shawkey, M.D. (2012) Relative contributions of pigments and biophotonic nanostructures to natural color production: a case study in budgerigar (*Melopsittacus undulatus*) feathers. Journal of Experimental Biology. 215, 1272 – 1277.

Delacour, J. (1942) Bird breeding in France. *Avic. Mag.* 1.

DeNise, S., Johnston, E., Halverson, J., Marshall, K., Rosenfeld, D., McKenna, S., Sharp, T., Edwards, J. (2003) Power of exclusion for parentage verification and probability of match for identity in American kennel club breeds using 17 canine microsatellite markers. *Animal Genetics,* **35**, 1, 14 – 17.

Diega, A. (2017) Bird flu to increase foreign demand for African lovebirds. Business Mirror. https://businessmirror.com.ph/bird-flu-to-increase-foreign-demand-for-african-lovebirds/

Dilger, W. (1960) The comparative ethology of the African parrot genus Agapornis. *International Journal of Behavioral Biology Ethology*. 17, 6, 649-685.

Dyck, J. (1971 a) Structure and colour-production of the blue barbs of *Agapornis roseicollis* and *Cotinga maynana*. Z. Zellforsch. 115, 17 – 29.

Dyck, J. (1971 b) Structure and spectral reflectance of green and blue feathers of the rose-faced lovebird (*Agapornis roseicollis*). *Det. Kongelige Danske Videnskabernes Selskab, Bilogiske Skrifter* 18, 2.

Eberhard, J.R. (1998) Evolution of Nest-Building Behavior in Agapornis Parrots. *The Auk,* 115, 2 455-464.

Egwumah, F.A. & Iboyi, M.O. (2017) Paramount factors influencing the breeding performance of lovebird *Agapornis pullaria*. *IJLST*, 10, 7, 69-74.

Elliot, F.S. (2013) Budgerigar matings and colour expectations. Read Books Ltd. United Kingdom.

Forshaw, J. (1989) Parrots of the World. Princeton University Press, United States of America.

Ganeshkumar, G. Howard, J.T., Ward, J.M., Li, J., Li, B., Li, Y., Xiong, Y., Zhang, Y., Zhou, S., Schwartz, D.C., Schatz, M., Aboukhalil, R., Fedrigo, O., Bukovnik, L., Wang, T., Wray, G., Rasolonjatovo, I., Winer, R., Knight, J.R., Koren, S., Warren, W.C., Zhang, G., Phillippy, A.M., Jarvis, E.D. (2014) High-coverage sequencing and annotated assemblies of the budgerigar genome. *GegaScience*, 3, 11.

Guay, P.-J., Potvin, D.A. & Robinson, R.W. (2012) Aberrations in plumage coloration in birds. *Australian field ornithology*, 29, 23 – 30.

Gunnarsson, U., Hellström, A.R., Tixier-Boichard, M., Minvielle, F., Bed'hom, B., Ito, S., Jensen, P., Rattink, A., Vereijken, A., Andersson, L. (2007) Mutations in *SLC45A2* cause plumage color variation in chicken and Japanese quail. *Genetic* 175, 857 – 577.

Harris, R.L. (1979) Genetics and the budgerigar. *AFA Watchbird Journal of the American Federation of Aviculture,* 6, 2.

Hayward, J. (1979) Lovebirds and their Colour Mutations.Blandford Press Ltd, United Kingdom.

Hill, G.E. & McGraw, K.J. (2000) Bird Coloration Mechanisms and measurements, Volume I. Harvard University Press, United States of America.

Hohenlohe, P.A., Amish, S.J. Catchen, J.M., Allendorf, F.W., Luikart, G. **(**2011) Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. *Molecular ecology resources*, 11, (Suppl. 1), 117 – 122.

Hudon, J. & Brush, A.H. (1990) Identification of carotenoid pigments in birds. *FASEB J*, 4, 2969.

Hume, J.P., & van Grouw, H. (2014) Colour aberrations in some extinct and endangered birds. *Bull. B.O. C.* 134, 3, 168 – 193.

Israel, C. & Weller, J.I. (2000) Effect of misidentification on genetic gain and estimation of breeding value in dairy cattle populations. *Journal of Dairy Science,* 83, 1, 181 – 187.

Jan C, Fumagalli L. **(**2016) Polymorphic DNA microsatellite markers for forensic individual identification and parentage analyses of seven threatened species of parrots (family Psittacidae). *PeerJ* 4.

Koutsogiannouli, E.A., Moutou, K.A., Sarafidou, T., Stamatis, C., Mamuris, Z. (2010) Detection of hybrids between wild boars (Sus scrofa scrofa) and domestic pigs (*Sus scrofa f. domestica*) in Greece, using the PCR-RFLP method on melanocortin-1 receptor (MC1R) mutations. *Mammalian Biology*, 75, 1, 69 – 73.

Manegold, A. & Podsiadlowski, L. (2014) On the systematic position of the Black-collared Lovebird *Agapornis swindernianus* (*Agapornithinae*, Psittaciformes). *J Ornithol*, 155, 581 – 589.

Marez, C. (2003) The Abyssinian lovebird Agapornis taranta. *AFA Watchbird Journal of the American Federation of Aviculture***,** 30, 3.

Matukumalli, L.K., Lawley, C.T., Schnabel, R.D., Taylor, J.F., Allan, M.F., Heaton, M.P., O'Connoll, J., Moore, S.S., Smith, T.P.L., Sonstegard, T.S., van Tassell, C.P. (2009) Development and characterization of a high density SNP genotyping assay for cattle. *PLOS One*, 4, 4.

McCarthy, E. (2006) Handbook of Avian Hybrids of the World. Oxford University Press, United Kingdom.

McGraw, K.J. & Nogare, M.C. (2005) Distribution of unique red feather pigments in parrots. *Biol Lett.* 1, 38 – 43.

McGraw, K.J. (2006) Mechanics of uncommon colors: pterins, porphyrins, and psittacofulvins. In: Bird Coloration Mechanisms and measurements, Volume I. Harvard University Press, United States of America.

Moreau, R. (1948) Aspects of evolution in the parrot genus *Agapornis*. *IBIS*, 90, 2, 206-239.

Mundy, N.I. (2006) A window on the genetics of evolution: MC1R and plumage colouration in birds. Proc. R. Soc. B. 272, 1633 – 1640.

Mundy, N.I. (2018) Colouration genetics: Pretty polymorphic parrots. *Current biology,* 26, 3, R113-R114.

Mwangomo, E.A., Hardesty, L.H., Sinclair, A.R.E., Mduma, S.A.R., Metzger, K.L. (2007) Habitat selection, diet and interspecific associations of the rufous-tailed weaver and Fischer's lovebird. *Afr. J. Ecol*, 46, 267 – 275.

Mzumara, T.I., Perrin, M.R. & Downs, C.T. (2016 a). The drinking habits of the Lilian's lovebird and incidents of poisoning at waterholes. *African Journal of Ecology*. 54, 3, 289 – 298.

Mzumara, T.I., Perrin, M.R. & Downs, C.T**.** (2016 b) Comparative performance of four survey methods for assessing Lilian's Lovebird abundance in Liwonde National Park, Malawi. *Ostrich*. 87, 3, 247 – 253.

Nanda, I., Karl, E.K Griffin, D.K., Schartl, M., Schmid, M. (2007) Chromosome repatterning in three representative parrots (Psittaciformes) inferred from comparative chromosome painting. *Cytogenet Genome Res* 117, 43 – 53.

Ndithia, H., Perrin, M.R., Waltret, M. (2007) Breeding biology and nest site characteristics of the Rosy-faced Lovebird *Agapornis roseicollis* in Namibia. *Ostrich*, 78, 1, 13 – 20.

Parkes, C. (1982) Avian Biology, Volume 6. Elsevier Inc.

Perrin, M.R. (2009) Niche separation in African parrots. pp. 29–37. In: Harebottle, D.M., Craig, A.J.F.K., Anderson, M.D., Rakotomanana, H. & Muchai, M. (eds). Proceedings of the 12th Pan-African Ornithological Congress, 2008. Cape Town, Animal Demography Unit.

Perrin, M. (2012) Parrots of Africa, Madagascar and the Mascarene islands. Biology, ecology and conservation. Wits University Press, South Africa.

Proceedings of the Zoological Society of London. 1932. Mutant of *A. personata*. 1418.

Prum, R.O., Morrison, R.L. & Ten Eyck, G. R. (1994) Structural color production by constructive reflection from ordered collagen arrays in a bird (*Philepitta castanea*: Eurylaimidae). *Journal of Morphology*, 222, 61 – 72.

Prum, R.O. (2006) Anatomy, physics, and evolution of structural colours. In: Bird Coloration Mechanisms and measurements, Volume I. Harvard University Press, United States of America.

Rasmussen, R.S. & Morrissey, M.T**.** (2008) DNA-based methods for the identification of commercial fish and seafood species. *Comprehensive reviews in food science and food safety*, 7.

Rheindt, F.E. & Edwards, S.V. (2011) Genetic introgression: an integral but neglected component of speciation in birds. *The Auk*, 128, 4, 620 – 632.

Sage, B.L. **(**1962) Albinism and melanism in birds. *British Birds*, 55, 6.

Silva T & Kotlar, B. (1988) Breeding Lovebirds. T.F.H. Publications, Inc., United States of America.

Snyders, N. F., Derrickson, S. R., Beissinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D., Miller, B. (1996) Limitations of captive breeding in endangered species recovery. Conservation biology, 10, 2, 338-348.

Stoddard, M.C. & Prum, R.O. (2011) How colorful are birds? Evolution of the avian plumage color gamut. *Behavioral Ecology*, 22, 5, 1042 – 1052.

Stradi, R., Pini, E., Celentano, G. (2001) The chemical structure of the pigments in *Ara macao* plumage. *Comparative Biochemistry and Physiology* Part B, 130, 57-63.

Tekalign, W. & Bekele, A. (2011) Status of and threats to the Black-winged Lovebird (*Agapornis taranta*) in Entoto Natural Park and Bole sub-city, Addis Ababa, Ethiopia. *Chinese Birds*, 2, 4, 174 – 182.

Toerien, M.J. (1950) On the change of colour in a rosy-faced lovebird. *Ostrich*, 21, 1, 19 – 21.

Tsudzuki, M. (2008) Mutations of Japanese Quail (*Coturnix japonica*) and recent advances of molecular genetics for this species. *The Journal of Poultry Science*, 45, 159 – 179.

Van den Abeele, D. (2016) Lovebirds Compendium, Genus Agapornis. About Pets, The Netherlands.

Van der Zwan, H., van der Westhuizen, F., Visser, C & van der Sluis, R. (2018) Draft de novo genome sequence of *Agapornis roseicollis* for application in avian breeding. *Animal Biotechnology*. In press.

Van Grouw, H. (2013) What colour is that bird? The causes and recognition of common colour aberrations in birds. *British Birds*, 106, 17 – 29.

Van Holdt, B.M., Pollinger, J.P., Earl, D.A., Parker, H.G., Ostrander, E.A., Wayne., R.K. (2013) Identification of recent hybridization between gray wolves and domesticated dogs by SNP genotyping. *Mamm genome*, 24, 80 – 88.

Visser, C., van Marle-Köster, E., & Friedrich, H. (2011) Parentage verification of South African Angora goats, using microsatellite markers. *South African Journal of Animal Science*, *41*(3), 250-255.

Veeckmans, D. (2016) New mutation of Fischer's lovebird – *Agapornis fischeri:* Opaline Euwing. Parrots Daily News. http://www.parrotsdailynews.com/new-mutation-of-fischers-lovebird-agapornis-fischeri-opaline-euwing/

Warburton L.S. & Perrin, M.R. (2005) Nest-site characteristics and breeding biology of the Black-cheeked Lovebird *Agapornis nigrigenis* in Zambia. *Ostrich.* 76, 3&4, 162 – 174.

Warburton L.S. & Perrin, M.R. (2006) The Black-cheeked Lovebird (*Agapornis nigrigenis*) as an agricultural pest in Zambia**.** *Emu* 160**,** 4, 321 – 328.

Weinman, L.R., Solomon, J.W., Rubenstein, D.R. (2015) A comparison of single nucleotide polymorphism and microsatellite markers for analysis of parentage and kinship in a cooperatively breeding bird. *Molecular Ecology Resources*. 15, 502 – 511.

Yakovlev AA, Kudryavtsev IV and Pigareva MD. (1975) A mutant Japanese quail (*Coturnix coturnix japonica*) with marble plumage. *Soviet Genetics*, 11: 1225 – 1232.