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Classification and Temporal Stability of Plumage Variation in Common Buzzards

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Classification and temporal stability of plumage variation in Common Buzzards

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Persistent plumage colour polymorphism occurs in around 3.5% of bird species, with raptors showing a disproportionately high frequency of such polymorphisms. The genus *Buteo* has more polymorphic species than any other raptor genus (15 polymorphic species out of 25). These polymorphisms are interesting from an evolutionary perspective, because they are heritable and hence a good model for understanding mechanisms preserving genetic variation. For evolutionary models, it is important to assess whether discrete morphs exist or whether variation is more continuous. Using image analysis, we show that in Common Buzzards *Buteo buteo* variation is continuous and unimodal, ranging from very dark to very light individuals. Previous studies on Common Buzzards have used a classification with three discrete morphs. We compared this classification with a seven-scale morph classification used in our study. We used photographs of the same individuals taken at different ages. Even though the plumage gets somewhat darker from juvenile to adult age, morph type did not change substantially.

Key words: *Buteo buteo*, Common Buzzard, colour polymorphism, plumage variation, morph, image analysis

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Investigations into the adaptive functions of animal coloration are widespread in behavioural and evolutionary biology. Studies investigating colour dominate functional and evolutionary investigations of camouflage, aposematism, mimicry, and both sexual and social signalling. Variation in coloration between individuals of the same sex and age, referred to as colour polymorphism, is found in many species throughout the animal and plant kingdoms (e.g. Jones *et al.* 1977, Kay 1978, Hoffman & Blouin 2000, Ferguson-Lees & Christie 2001). In birds, plumage colour polymorphism is found in 3.5% of all bird species (Galeotti *et al.* 2003). Polymorphisms are relatively common among raptors compared to other taxa, with 30% of raptors showing some polymorphism (Fowlie & Krüger 2003,

Hugall & Stuart-Fox 2012). Plumage coloration can vary continuously or may show two or more discrete morphs: for example, the polymorphic Swainson's Hawk *Buteo swainsoni* shows continuous variation in plumage colour, although it is often classified as dark, light or intermediate for analyses (Briggs *et al.* 2011). In contrast, a discrete polymorphism exists e.g. in the Eleonora's Falcon *Falco eleonorae* (Gangoso *et al.* 2011) and the Black Sparrowhawk *Accipiter melanoleucus* (Amar *et al.* 2013), with either dark or light morph birds.

Common Buzzards *Buteo buteo* have been used as a model to study the maintenance of genetic colour variation (Krüger *et al.* 2001), but morphs have been poorly characterized in the literature because they

are not unambiguously defined. Based on 63 museum specimens, Ulfstrand (1970) described a spectrum from dark brown to very pale by subdividing the bird's plumage into 16 parts and calculating an index for the overall pigmentation. His results showed that variation in pigmentation is much greater on the underwings and on the ventral side than on the dorsal side. Ulfstrand (1970) states that "the frequency curve of the total pigmentation indices of the sample examined does not reveal any trace of bimodality, as would have been expected if the variation had been discontinuous and the population divided into two distinct colour phases", but unfortunately he did not provide the data nor any statistics.

In contrast, Krüger and colleagues (Krüger *et al.* 2001, Krüger 2002, Chakarov *et al.* 2015, 2017), studying Buzzards in Germany, refer to Glutz von Blotzheim *et al.* (1971) and Melde (1983) and distinguish between three morph types: (1) light: little or no melanisation of breast and underwing coverts, (2) intermediate: dark head, intermediately speckled breast and underwing coverts, and (3) dark: dark head, heavily speckled or dark breast and underwing coverts. Using this simple classification, Krüger *et al.* (2001) demonstrated that morph inheritance generally follows Mendelian expectations for a single locus with two, presumably co-dominant, alleles.

Another study (Dittrich 1985) described five morph categories: "Morph 1: under- and upperparts, including head, wings and tail, dark, without clearly visible pattern; morph 2: upperparts more or less uniform dark, pattern on underparts; morph 3: upperparts like 2, pattern on pale underparts strongly reduced or lacking; morph 4: upperparts with very large pale parts, underparts like 3; morph 5: upper- and underparts, without pattern, extremely light (primary tips are always dark, distal bar[s] on tail more or less pigmented)".

The different scoring systems used by these authors could be idiosyncratic, but they may also reflect how variable the plumage is at a particular study site, and/or how relative frequencies of the morphs vary between their respective study populations. Different morph-composition of populations across Europe could lead to different categorization scales. Clearly, when studying general biological processes to explain the maintenance of colour variation in this species, it would be helpful if scoring systems were comparable between studies. Besides the practical issue of comparing results between studies, continuous variation in plumage colour would be harder to reconcile with a simple one-locus, two-allele inheritance pattern.

For adult (Buteonine) hawks, it has often been assumed that plumage morph is invariant over time, i.e. the plumage pattern does not change as an individual ages. Briggs *et al.* (2010) examined this in their population of Swainson's Hawks and their results indeed indicate that an individual's plumage does not change for up to 17 years. Krüger and colleagues assume a similar pattern in the Common Buzzard as the basis to identify individuals across years (e.g. Krüger *et al.* 2001), but this has given rise to some criticism (Roulin, 2004), because the literature does not provide unequivocal evidence for the Common Buzzard.

Common Buzzards undergo a single pre-basic moult each year, (mainly) during the breeding season. There is no postjuvenile moult, so that birds retain the feathers they acquire in the nest until the following spring. Common Buzzards have 'Juvenile' plumage during the first moult cycle (first year of age), followed by 'Second' and 'Third Basic' plumage during the second/third moult cycle, until 'Definitive Basic' plumage in the fourth moult cycle. The latter is the plumage that supposedly remains unchanged from year to year and is found in most breeding adults. Prytherch (2009) states that "plumage variation is such that some juveniles (especially dark ones) can look very similar to older birds" and suggests that plumage may change progressively with age in adult Buzzards. However, there are no data available to support this.

The first aim of this paper is to describe quantitatively the colour variation in the plumage of Common Buzzards with the use of digital photography and image analysis. We then compare the quantitative description with a seven-scale scoring system to assess whether qualitative categorization is a reasonable description of variation in plumage coloration. Finally, we compare different qualitative scoring systems of plumage coloration in the Common Buzzard to assess how studies can be replicated. The second aim of our study is to test whether the morph type based on the juvenile plumage (which can be scored when the individual is still in the nest, just before fledging) is a good predictor for adult morph in the Common Buzzard. If morphs are invariant with age, this allows investigation of the genetic inheritance of the colour morph of both parents and their offspring just before they fledge.

METHODS

Image analysis

To quantify plumage colour variation, we carried out an image analysis using photographs of Common

Figure 1. Cumulative frequency distribution of the pixel values (0 = black, 255 = white) of original and standardized photographs (solid and dashed lines respectively) of three different morph types. Stars refer to the standardized median pixel value for each bird on the right. Black curves: upper photo (morph-score 'dark-intermediate', standardized median = 55), dark grey curves: middle photo (morph-score 'intermediate', standardized median = 85), light grey curves: bottom photo (morph-score 'very light', standardized median = 139).

Buzzards caught in the field with a bal-chatri trap. Digital photos (JPEG files) were taken of birds in juvenile and adult plumage after being caught for ringing. The Buzzards were caught opportunistically during the non-breeding season (September–March) when food is often scarce and Buzzards are more easily attracted to live bait in the trap. Trapping sessions were carried out in areas where the population was monitored for many years, so individuals caught could be resident birds (recaptures) or unknown individuals only wintering. Photos of trapped individuals were usually taken from the front with the wings held open. Only photos where the bird was homogeneously illuminated (no sunlit versus shadowed patches) and that clearly showed the front and at least one underwing were used for scoring coloration. In total, photos from 93 individuals were used, of which 74 are from individuals of known age (range: 1–27 years, median = 5.7; based on plumage pattern – juveniles have more immaculate plumage with marks consisting mostly of streaks and tail without distinct terminal band (Cramp & Simmons 1980) – and iris characteristics for juveniles and subadults, or on recapture data for adults ringed as nestlings) and 54 of known sex (32 males and 22 females, based either on size differences of individuals ringed in the nest or on behavioural information from the breeding season). Information on both age and sex was available for 45 individuals.

Plumage coloration from the 93 individuals was scored both qualitatively and quantitatively to compare the two methods. First, one observer (EFK) scored the

morph according to the seven-scale morph classification system described below. Second, we processed the images before measuring the coloration quantitatively with R (v. 3.3.1; R Core Team 2016).

Because no colour standard was used in the photos, we could not directly compare the relative plumage coloration and hence only compared the amount of unpigmented versus pigmented plumage in each image as follows. (1) We grey-scaled each image, i.e. we created an image with values from 0 (black) to 255 (white). (2) We 'cut out' the Buzzard and removed the background. (3) We rotated the body so that the wings were aligned in the same orientation in all pictures (Figure S1). (4) With the R package 'raster' (Hijmans 2016) we black-balanced the pixels to correct for different light conditions in the photos as follows: we used the wing tip as reference for black, and obtained the reference value from the fifth percentile of pixels in the wing tip (Figure S1), and to standardize each photo, we black-balanced by subtracting the reference value from all pixels. (5) We used the median pixel value after standardization as an index of individual coloration (see example in Figure 1).

First, we tested for multimodality in the frequency distribution of the colour index of the 93 individuals using Hartigan's dip test for unimodality (Maechler 2015). Then, we tested whether the colour index obtained from the image analysis correlated with the qualitative seven-morph categories by using a linear model. We also tested whether colour was age- or sex-dependent, using two linear models, one with the

Figure 2. Example photos illustrating the seven-morph categorization from very dark to very light. (1) Very dark: 0–2% light plumage on the chin, throat, breast and flanks (hereafter front) and underwing coverts. (2) Dark: individuals are overall dark with a little white, having 3–10% light plumage on the front and underwing coverts. (3) Dark-intermediate: individuals can have a streaked chest or a pale breast band, but the head, collar and belly are still dark, making the percentage of light plumage on the front and underwing coverts overall between 11 and 35%. (4) Intermediate: individuals have between 36 and 60% of light plumage both on the underwing and frontal parts of the bird. Pale barred breast and belly are typical, with dark head and flanks. (5) Light-intermediate: the neck can be light and spotted, with a lighter streaked head, but the flanks are still brown. The percentage of pale feathers ranges between 61–80% of the underwing and frontal body. (6) Light: individuals are overall pale (81–100% of light plumage on underwing and frontal part of the body), but can have some brown on the head and neck. Underwings are pale, but upperwing is still brownish. (7) Very light: distinguished from light individuals by having mainly pale upperwing coverts.

quantitative colour index and one with the qualitative morphs as the dependent variable, and age and sex (and their interaction) as independent variables.

Plumage scoring

In our long-term study of a population of Common Buzzards in Friesland, The Netherlands (53°04'09"N, 6°13'46"E), we described variation in plumage coloration using seven categories, as illustrated and described in Figure 2.

To compare our seven-scale scores with the three-morphs scoring system used by Krüger and colleagues

(see above), Oliver Krüger (OK), Nayden Chakarov (NC), Anna-Katharina Mueller (AKM) and Christiaan de Vries (CdV) scored 62 photos of soaring Common Buzzards and 64 photos of perching individuals, each following their scoring system (3 morphs for OK, NC and AKM, 7 morphs for CdV). Photos were chosen from the Dutch website for nature observations 'www.waarneming.nl' and all observers independently scored the same set of photos. Scoring was done immediately after a photo was shown on a screen, i.e. without comparing between photos. Photos were scored in the same order of appearance by all scorers.

Table 1. Classification of morph by percent of light plumage on the front and underwing coverts in 10 Common Buzzards from Friesland, The Netherlands. Individuals were trapped and photographed as juveniles and again after one or more cycles of moulting. Shown are means $\pm$ SD.

ID	Age	Date		% Light plumage				Morph	
				Front		Underwing			
		1 st photo	1 st photo	2 nd photo	1 st photo	2 nd photo	1 st photo	2 nd photo	1 st photo
A	1	08-02-2012	15-11-2012	46±11	29±8	42±12	38±14	Interm.	Dark- Int.
B	1	19-02-2012	29-12-2012	45±16	16±10	42±15	20±10	Interm.	Dark- Int.
C	1	06-03-2012	17-11-2012	38±14	28±8	48±18	42±13	Interm.	Dark- Int.
D	1	27-12-2011	22-02-2013	49±8	43±13	62±9	47±10	Interm.	Interm.
E	1	05-02-2012	10-03-2013	40±11	32±13	42±8	42±12	Interm.	Interm.
F	2	20-10-2012	13-10-2013	53±5	49±11	49±11	49±15	Interm.	Interm.
G	1	19-12-2011	06-12-2013	66±9	57±12	68±8	66±15	Light-Int.	Interm.
H	1	11-12-2011	07-03-2014	71±9	60±11	89±5	89±5	Light-Int.	Light-Int.
I	1	23-01-2012	28-12-2012	87±8	91±5	87±4	87±5	Light	Light
J	1	11-11-2012	01-11-2014	84±6	79±9	95±2	92±4	Light	Light

Figure 3. Paired photographs of 10 individual Common Buzzards from The Netherlands, taken first at juvenile age (left pictures) and after at least one moult cycle (right pictures). Letters refer to Buzzard ID shown in Table 1.

Figure 3. Continued

Consistency of the scoring with age

For 10 ringed juvenile Common Buzzards photographed in their first ($n = 9$) or second year ($n = 1$) a picture was also taken later in life (Table 1, Figure 3). These pairs of photos were used to assess the consistency of the scoring after one or more cycles of moulting. Because the wing tips were not always included in these photos, standardization with image analysis was not possible and hence visual scoring was used instead. Specifically, 13 observers visually scored the percentage of light plumage in two body parts (the front and the underwing coverts) in all photos. Photos were shown successively in random order and blind to individual identity. We ran a linear mixed model (with Gaussian distribution; package 'lme4'; Bates *et al.* 2015) with the given scores as response variable, body part, age and their interaction as fixed effects and both Buzzard and observer identity as random effects.

RESULTS

Quantitative description using image analysis

Our analysis of pictures from 93 individual Common Buzzards shows that the distribution of median pixel values as a quantitative colour index is continuous (Figure 4). The frequency of the extreme morphs (lowest and highest values) in our 'population' was lower compared to the frequency of intermediates, overall showing a close to normal distribution (median = 88.7, mean = 93). The Hartigan's dip test shows that the colour distribution does not significantly differ from a unimodal distribution ($D = 0.021$, $P = 0.99$).

Figure 4. Frequency distribution of non-transformed median pixel values as colour index (0 = black, 255 = white) for 93 individual Common Buzzards based on photographs of the front and underwing. The superimposed curve represents the frequency density distribution.

Figure 5. Relation between quantitative measurement and qualitative score of plumage coloration for 93 Common Buzzards. The line depicts the regression line ($r = 0.70$, $P < 0.01$). The colour index varies between black (0) and white (255). The morph score varies between very dark (1) and very light (7).

Median pixel values correlated strongly with scores in the seven-scale morph categories ($r = 0.70$, $P < 0.01$; Figure 5). Plumage colour, both when expressed quantitatively or qualitatively, did not differ between individuals of different age or between males and females (Figure 6, Table 2).

Comparison of scoring systems

Based on the scoring of 126 photos, we found a strong correlation among the three researchers that used the three-morph scoring (OK-NC: $r = 0.841$, OK-AKM: $r = 0.892$, NC-AKM: $r = 0.849$). We then used the median value of the three scores for each photograph and found that it also strongly correlated with the seven-morph scores from CdV ($r = 0.848$; Figure 7). However, the borders between the categories were not always coinciding among the two scoring systems. Specifically, the dark morph in the three-category system happened to include 100% of the very dark individuals, but only 60% of the dark individuals in the seven-category system (Figure 7).

Age effects

The photos of the same 10 individuals recaptured at intervals ranging from 8.5 to 35.9 months (mean = 18.7) show that the coloration as scored by 13 independent observers was rather consistent within individuals, both when considering the percentage of light plumage scored (front: repeatability $r = 0.747 \pm 0.108$ ($\pm$ SE); underwing: $r = 0.798 \pm 0.096$) or when comparing the morph categories (Table 1). However, the estimated percentage of light plumage decreased

Figure 6. Plumage colour of Common Buzzards expressed (A) qualitatively as morph score (1 = very dark, 7 = very light) and (B) quantitatively as median pixel value in relation to age for 74 individuals of known age (blue: males, pink: females; black: unknown sex; data points were jittered to avoid overlapping). Plumage colour was unrelated to age (see Table 2 for statistical details).

Table 2. Results of a linear model describing effects of age and sex on plumage coloration of 45 Common Buzzards, whereby colour was either defined as an index (median pixel value) or as morph (seven categories).

Variable	Estimate ± SE	t-value	P-value
Colour index			
Intercept	81.65 ± 10.42		
Age	0.79 ± 0.73	1.07	0.53
Sex ¹	-4.71 ± 9.79	-0.48	0.90
Morph			
Intercept	4.34 ± 0.54		
Age	0.02 ± 0.03	0.68	0.78
Sex ¹	-0.54 ± 0.50	-1.07	0.53

Estimated effect sizes of each term (Estimate) with associated standard errors (SE), *t*- and *P*-values are presented based on the minimal adequate model.
¹Estimate for males relative to females (= 0).

significantly with age (on average 6.1% fewer light feathers in older individuals) and this was stronger in the frontal part than in the underwing (significant interaction between age and body part in Table 3; Figure 8). For six individuals, the assessment of the morph score did not change, whereas for four individuals the morph changed to the next darker category in the seven-morph scale (Table 1).

Figure 7. Comparison of two morph-scoring systems for the categorization of 126 Common Buzzard photos. The Y-axis represents the percentage of photos scored dark, intermediate or light on the three-morph scale. The x-axis gives the morph on the seven-morph scale from very dark (1) to very light (7). *n* refers to the number of individuals (photos).

Figure 8. Changes in the percentage of light plumage with age for the front (A) and the underwing (B) in 10 Common Buzzards from Friesland, The Netherlands. Each square represents the mean score from 13 observers. Each individual is shown by a different line pattern and/or symbol. Individuals became significantly darker with age, and this effect was stronger for the front than for the underwing (see Table 3 for statistical details).

DISCUSSION

The Common Buzzard is one of several raptor species showing highly variable plumage within populations. To understand the biological significance of such plumage polymorphism requires a good description of this variation. We showed that Common Buzzards in The Netherlands display continuous variation in plumage coloration without evidence for multimodality, and hence no distinct morphs can be described. The observed variation is consistent within individuals (high repeatability), even though birds become somewhat darker with age. We detected no sexual difference in plumage colour. Because variation appears to be continuous, scoring systems containing more categories would capture the underlying variation better, and we suggest that the seven-morph scale describes this continuous colour variation reasonably well. As previous studies have used different scoring systems with fewer morphs, we show how our seven-morph scale relates to the previously described three-morph scale.

Field studies on bird species with variable plumage have often used a small number of distinct categories to describe the variation. Some species indeed have clearly defined polymorphisms, for example the Eleonora's Falcon (Gangoso *et al.* 2011) and the Black Sparrowhawk (Amar *et al.* 2013). However, in many other instances species were classified into several morphs, although coloration was known to vary continuously (e.g. Arctic Skua *Stercorarius parasiticus*, O'Donald 1983, Snow Goose *Anser caerulescens*, Cooke & Cooch 1968, Variable Oystercatcher *Haematopus unicolor*, Baker 1973). Categorisation of individuals

into a discrete number of morphs is often done for practical reasons, for example because of the difficulty of recording plumage traits on a continuous scale (see e.g. Cooke & Cooch 1968). The same is true for other Buteonine species such as the Swainson's Hawk, the Red-tailed Hawk *Buteo jamaicensis* and the Ferruginous Hawk *Buteo regalis*: despite more or less continuous variation from light to dark individuals, individuals have been assigned to discrete morph categories (Biggs *et al.* 2011, Palmer 1988, Preston 1980, Schmutz & Schmutz 1981). Our quantitative analysis of plumage coloration showed that Common Buzzards also express a continuous gradient with no distinctive multimodality (Figure 4). Nevertheless, we also consider categories that capture some of the continuous variation highly useful in field studies, because in many cases standardized photos are not available. It is rather arbitrary how many categories one should include, and this may depend on the specific purpose of the study. From our results, it seems that a seven-scale scoring system is a good qualitative descriptor of plumage coloration in the Common Buzzard, even though there was no clear separation between subsequent categories with some overlap in colour index ranges.

Studies addressing evolutionary questions about the maintenance of plumage variation require estimating both the inheritance of, and selection on the trait. In case of distinct, discrete colour morphs, one or a few genes are often involved that code for the variation (Mundy 2005). In this case, relatively simple models can be used to assess selection on the trait. In previous work on Common Buzzards, this approach has been fruitful, showing selection favouring intermediately coloured individuals, and suggesting that these were the heterozygotes in a one-gene, two-allele system (Krüger *et al.* 2001). However, continuous variation in plumage colour is often indicative of an underlying polygenic system of genetic control, where many genes with minor and cumulative effects are involved (Mather 1949). Thus, a pertinent question is whether including the more continuous variation observed in Common Buzzards would result in different conclusions. It is still possible that the selection dynamics can be understood when simplifying colour variation to three morphs, for example when there is one gene with a major effect and many with minor effects, resulting in continuous variation. This would require that the classification used in the studies by Krüger and colleagues (e.g. Krüger *et al.* 2001, Boerner & Krüger 2009, Chakarov *et al.* 2015, 2017) aligns well with the underlying variation in the major gene. To test assumptions about the underlying genetic mechanism, it is impor-

Table 3. Results of a linear mixed model describing effects of age and body part on plumage coloration of 10 Common Buzzards measured twice. Plumage coloration was scored as percentage of light plumage.

Variable	Estimate $\pm$ SE	z-value	P-value
Intercept	63.87 $\pm$ 7.30		
Age	-6.13 $\pm$ 0.90	6.78	<0.001
Body part ¹	1.45 $\pm$ 2.30	0.62	0.89
Age $\times$ Body part ¹	3.01 $\pm$ 1.20	2.49	0.03

Estimated effect sizes of each term (Estimate) with associated standard errors (SE), z- and P-values are presented based on the minimal adequate model.

¹Categorical variable (front/underwing). Front is reference category (= 0).

Observer ($n = 13$) and Buzzard ($n = 10$) identity were included in the model as random effects.

tant that models with different inheritance models are compared, as was done for example in the Tawny Owl *Strix aluco* (Karell *et al.* 2011).

We found a strong correlation between scores based on the three-morph scoring system used by Krüger and colleagues on a German population and a seven-morph scale developed by Christiaan de Vries on a Dutch population (Figure 7). However, individuals assigned to the 'light' category in the three-morph system ended up in four categories in the seven-morph scale, whereas all but one 'dark' individual ended up in two categories (Figure 7). The establishment of classification scales may be influenced by the relative frequencies of morphs in the study area considered, and therefore scoring systems may differ between study areas and research teams. Given that differential distributions of morphs have already been described for other polymorphic raptors (Amar *et al.* 2014, Antoniazza *et al.* 2010), we cannot exclude that a geographical factor could play a role in the definition of the morph scale for Common Buzzards. For this raptor, remarkably little is known about the geographical distribution of the morphs (Ulfstrand 1977). Therefore, we launched the "Buteo Morph" project where citizen scientists can enter their sightings and classify individuals on a seven-morph scale in order to map morph distribution for the Common Buzzard on a large scale (<http://aves.orn.mpg.de/~buteo/en>).

Investigating the potential inheritance patterns of colour variation in Common Buzzards should be relatively straightforward, as we have shown that plumage colour at fledging does not seem to change substantially as individuals age. Thus, plumage colour of offspring in juvenile plumage can be directly compared with that of their parents in definitive basic plumage. On average, juveniles became about 6% darker in the following moult cycle(s), whereby the change in plumage coloration with age was more visible on the front part of the body than on the underwing (Figure 8). We also showed that plumage coloration, scored quantitatively or qualitatively for 93 individuals, did not differ significantly between males and females or in relation to age. This confirms that the colour polymorphism is sex-independent. Indeed, the Common Buzzard belongs to the 55% polymorphic raptor species that show no sexual dimorphism in plumage coloration (Ferguson-Lees & Christie 2001). Our results also confirm the long-standing assumption that plumage morph of Common Buzzards is invariant over time, i.e. does not change substantially when individuals become older (e.g. Krüger *et al.* 2001). Similar findings were published for other raptor species (e.g. Swainson's

Hawk, Briggs *et al.* 2010, Black Sparrowhawk, Amar *et al.* 2013, Tawny Owl, Brommer *et al.* 2005).

In conclusion, this study shows that even though plumage coloration in Common Buzzards is continuous – which can be difficult to score in the field – a seven-morph categorization captures the continuous variation well. This scoring system will be a useful tool to address evolutionary questions about the maintenance of the colour polymorphism, using data on fitness components measured under natural conditions. Our future research will focus on estimating heritability of the continuous plumage variation using a quantitative genetics approach to show how additive and non-additive genetic effects underpin coloration in this species, and on evaluating the likelihood of a one locus two-allele inheritance pattern.

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SAMENVATTING

Bij ongeveer 3,5% van alle vogelsoorten zien we meer dan één permanent type verenkleed (polymorfisme). Roofvogels vertonen een onevenredig hoge frequentie aan polymorfisme. Het geslacht *Buteo* heeft meer polymorfe soorten dan welk ander roofvogelgeslacht ook (15 van de 25 soorten van dit geslacht zijn polymorf). Vanuit een evolutionair perspectief zijn deze polymorfismen interessant, omdat ze erfelijk zijn en dus een goed model vormen voor het begrijpen van mechanismen die zorgen voor het behoud van genetische variatie. Voor evolutionaire modellen is het belangrijk om te kunnen vaststellen of er discrete kleurvormen (geen tussenvormen) bestaan of dat de variatie meer continu is. Met behulp van beeldanalyse konden we aantonen dat bij Buizers *Buteo buteo* de variatie continu en unimodaal is, variërend van zeer donkere tot zeer lichte individuen. Bij eerdere studies aan Buizers is gebruikgemaakt van een classificatie met drie discrete kleurvormen. We vergeleken deze classificatie met de zevenschalige classificatie die wij bij ons onderzoek hebben gebruikt om in het vervolg een betere vergelijking te kunnen maken tussen de verschillende studies. Ook onderzochten we met behulp van foto's die gemaakt waren van dezelfde individuen op verschillende leeftijden, hoe sterk kleurvariatie verandert tijdens het leven. We vonden dat het verenkleed van juveniele vogels iets lichter was dan op latere leeftijd, maar dat deze verandering nauwelijks effect had op het kleurtype binnen de zeven gebruikte schaalklassen.

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SUPPLEMENTARY MATERIAL

Figure S1. Example of one of the 93 photos being grey-scaled (0 = black, 255 = white) and rotated before image analysis. The vertical dashed line marks the right 5% of the photo length that was used to define the reference black colour.