Polymorphic Common buzzards in time and space

Elena F. Kappers

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Polymorphic Common buzzards in time and space

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by

Elena Frederika Kappers

born on 6 September 1988
in Apeldoorn

Supervisors

Prof. C. Both
Prof. B. Kempenaers

Assessment Committee

Prof. T. Piersma
Prof. M.E. Maan
Prof. J.E. Brommer

Voor Opa en Oma Kappers,

Contents

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General introduction

Elena Frederika Kappers

The rich morphological and behavioural variety of traits in the natural world is the result of the evolutionary history of species and populations. The identification of the processes maintaining both phenotypic and genetic variability in wild populations is a major challenge in evolutionary biology. In this thesis, I investigated the evolutionary ecology of colour variation in a bird species.

Evolution is defined as the change in genotype frequencies in populations over successive generations. The striking diversity of morpho-behavioural traits that can be observed in the living world can be understood considering the following three main evolutionary principles: the first is that variation must exist in the traits; the second, is that variation in the traits must be heritable; the third, is that some traits must have higher chance to be passed to the next generation (Darwin 1859). When the characteristics of organisms are beneficial to the reproduction of individuals within a given environment- i.e., they increase the "fitness" of the individual- copies of the allelic forms of the genes that are responsible for these characteristics are more likely to be inherited by the next generation. The consequence is an increase in the frequency of these characteristics within the population over time (and consequently a decrease in frequency of alternative alleles).

Measures of individual (phenotypic) fitness are implicitly substituted for measures of genotypic fitness in most studies. Individual fitness designates the success of a phenotypic trait within one generation. It corresponds then to the average demographic success of a phenotype relative to the success of other phenotypes present in the population. The quantification of individual fitness can be limited to a short period in the life of an individual (e.g. winter survival or yearly number of offspring produced) or, ideally, according to the total reproductive success of an individual calculated over its entire lifetime. Fitness is not constant in natural populations (Kojima 1971), but is likely to change under different environmental conditions. Fitness may also change when allele frequencies change, which is called frequency-dependent selection (Smith and Price 1973).

In this thesis, I quantified individual fitness in a diurnal raptor with very variable plumage coloration, the Common buzzard *Buteo buteo*. I attempt to unravel some of the mechanisms that maintain intra-specific colour variation (hereafter colour polymorphism) and its functions in this species. I studied colour polymorphism from both a temporal and a spatial perspective. I first present a short introduction on colour polymorphism in avian species, followed by an overview on the putative mechanisms for its maintenance and finally I review the previous research done on my model species.

Colour polymorphism

Colour polymorphism (first defined by Ford 1945) has long captivated evolutionary biologists. Visible polymorphisms are widespread in plants and animals and have been used as an excellent model system to examine micro-evolutionary processes (Jones et al. 1977; Kay 1978; Hoffman and Blouin 2000; Roulin 2004b; Gray and McKinnon 2007; Forsman et al. 2008). Among animal species, the incidence of colour polymorphism appears higher in birds, anurans and lepidopterans (Hoffman and Blouin 2000; Roulin 2004b).

In birds, plumage colour polymorphism can be found in 3.5% of all bird species (Galeotti et al. 2003), and it's widespread in many different orders such as hawks, eagles, kites, Old World vultures, owls, nightjars, falcons and pheasants (figure 1.1). In the Accipitridae, Striginae, Surniinae and Caprimulgidae colour polymorphism is most prevalent (>20% of species). Many colour polymorphisms have a simple genetic basis and show high heritability (Mundy 2005).

Morphs that coexist at relatively stable frequencies appear to be common in natural environments (e.g. Gray 1983; Reillo and Wise 1988; Franklin and Dostine 2000; Honěk et al. 2005). Nevertheless, the mechanisms that underpin morph evolution and maintenance are often poorly understood in the wild. Some of the more common mechanisms proposed are frequency-dependent selection, hetero-zygote advantage, and genotype-by-environment interactions.

Frequency-dependent selection is an evolutionary process by which the fitness of genotypes (or phenotypes) depends on their frequency in the population. It can favour phenotypes that are either common (positive frequency-dependent selection) or rare (negative frequency-dependent selection). This form of selection has been used to explain the maintenance of colour polymorphisms in a number of species (Hori 1993; Bond and Kamil 1998; Takahashi et al. 2010). An interesting example of this type of selection is seen in side-blotched lizards (*Uta stansburiana*). Males come in three throat-colour patterns: orange, blue, and yellow. Each of these forms has a different reproductive strategy and like a game of rock-paper-scissors, orange beats blue, blue beats yellow, and yellow beats orange in the competition for females. As a result, populations of side-blotched lizards cycle in the distribution of these phenotypes (Sinervo and Lively 1996). Another remarkable example of negative frequency-dependent selection is apostatic selection, where the rare morph prey animals is more likely to be ignored by their predator than the common morph, giving the rare morph a selective advantage in the population. Apostatic selection has been used to explain morphs in predator-prey systems (Paulson 1973; Bond and Kamil 1998).

Heterozygote advantage describes the case where heterozygous individuals have a fitness advantage and has been proposed as a mechanism for sickle cell anaemia in humans (Allison 1964), sperm design in Zebra finches *Taeniopygia guttata* (Knief et al. 2017) and plumage coloration in Common buzzards (Krüger et al. 2001).

For these two mechanisms, we can predict the direction of fitness for the morphs present in a species. In apostatic selection, fitness is highest for the rarest morph type(s), which could lead to changes in morph frequencies over time. Specifically, at the equilibrium frequency, the fitness of morphs should be equal. Thus, the rare morph will only have increased fitness up to an equilibrium. In contrast, heterozygote advantage predicts that in heterozygotes at least some component of fitness should be higher than either homozygous state regardless of frequency in the population.

Figure 1.1: Few representative examples of colour polymorphic raptors: (**a**) light and dark morph of the Eleonora's falcon *Falco eleonorae*, (**b**) pale and rufous morph of the Barn owl *Tyto alba*, (**c**) the two colour morphs of the Tawny owl *Strix aluco*, (**d**) the light and dark morph of the Black Sparrowhawk *Accipiter melanoleucus,* (**e**) light, intermediate and dark morphs of the Swainson's hawk *Buteo swainsoni*.

Colour polymorphisms may also be maintained by genotype-by-environ-ment interactions, where some genotypes are selectively favoured in certain habitat types (Gillespie and Turelli 1989). The local environment plays a large role in shaping phenotypic differences across the distributional range of a species, both in its proximate effect on traits through phenotypic plasticity and its ultimate impact on the evolution of local adaptations. The latter arises as a result of selection by the local environment favouring phenotypes that have a higher chance to survive and reproduce (e.g. Dreiss et al. 2012). Geographic patterns of phenotypic variation of populations of the same species are in part due to this selective process (Antoniazza et al. 2010; Amar et al. 2014).

There are many examples in nature on fitness differences in polymorphic species. In studies that have examined ecological differences between colour morphs, it is not uncommon to find differences in life-history traits (i.e. probabilities of survival and rates of reproduction at each age in the life-span) or in lifetime reproductive success (Roulin 2004b). For example, reproductive parameters covaried with genetic colour polymorphism in a number of species: O'Donald (1983) found differential age at first reproduction and hatching date in polymorphic Artic Skuas *Stercorarius parasiticus*; Brommer et al. (2005) showed differential lifetime production of fledglings and recruits in the brown and grey morphs of the Tawny owl; Saino and Bolzern (1992) found differential hatching success in morphs of the Carrion/Hooded crow *Corvus corone corone*/*cornix*. Johnson and Burnham's (2013) study on Gyrfalcons *Falco rusticolus* in Greenland, found differential egg laying date and production of offspring among the three morphs. Krüger and colleagues (2001) found that intermediate morphs of Common buzzards display a higher lifetime reproductive success compared to the extreme morphs. In Swainson's hawks *Buteo swainsoni*, however, Briggs et al. (2011) found no differences in productivity or life-time reproductive success among morphs.

Differences in adult survival across morphs have been described in Tawny owls (Brommer et al. 2005; Karell et al. 2011) and Lesser snow geese *Chen caerulescens caerulescens* (Cooch 1961). Amongst diurnal raptors however, very few studies have examined survival rates across morphs. For example, Krüger et al. (2001) found differences in survival rates between Common buzzard morphs. Using more robust methods, Jonker et al. (2014) found similar trends in the same buzzard population, but these differences in survival rates were only weakly supported. Interestingly, Briggs et al. (2011) found no support for differential survival across morphs in the Swainson's hawk.

In a range of species also differential spatial distribution has been described. In Barn owls (*Tyto alba*), morphs are adapted for a specific habitat type and show differences in diet (Roulin 2004a; Dreiss et al. 2012). Differential habitat selection has also been observed between morphs of Red-tailed hawks (*Buteo jamaicensis*, Preston 2009) and in the Bananaquit (*Coereba flaveol*, Wunderle Jr 1981). However, within species results often vary across studies indicating that the sign and magnitude of covariations between fitness parameters and polymorphism can vary in time and space.

Alternatively to the existence of fitness differences in polymorphic species, there may not be selective advantages or differences in life-history strategies that maintain different morphs within a population. Factors such as sexual selection (e.g. assortative mating) or large population size (Fowlie and Krüger 2003) may maintain multiple morphs within a population not requiring further explanations of fitness differences. Indeed, a number of studies have not found differences in components of fitness among morphs within populations (reviewed in Meunier et al. 2011).

The study system

The Common buzzard

The Common buzzard provides an interesting example for studying the maintenance of colour polymorphism because it is a widespread and common Eurasian raptor that exhibits an ostensibly continuous variation in plumage (figure 1.2) (Krüger et al. 2001; Boerner and Krüger 2009; Chakarov et al. 2016). Specifically, the coloration on the belly, flanks and underwing coverts ranges from very dark (melanic) to very light (figure 1.2) (Ulfstrand 1970, 1977). This variation is usually grouped in three main morphs by several authors: light, intermediate, and dark (Melde 1983; Blotzheim and Bauer 1997; Krüger et al. 2001). Several studies have shown that melanic polymorphic phenotypes in birds are genetically determined (Mundy 2005) and follow a Mendelian mode of segregation (Roulin 2004b). Krüger et al. (2001) argued that dark and light alleles show incomplete dominance and heterozygous individuals therefore display intermediate plumage between the two homozygous morphs, and hence give rise to continuous polymorphism along the plumage spectrum. However, the high variation in Common buzzards seems actually to be hardly compatible with a simple Mendelian inheritance pattern and variation may thus be controlled by several other genes.

Krüger and colleagues (2001; Boerner and Krüger 2009) found that the light and dark morphs have a much lower fitness than the presumed heterozygous intermediate morph, but are replenished through Mendelian segregation with the mating of intermediate phenotypes. In their German study population, intermediate individuals had higher survival, reproduction, reproductive value and lifetime reproductive success. Because the variation in these morphs has a genetic basis (Mundy 2005), the covariation between phenotype and fitness parameters can be considered as direct selection on the genetic component that controls the colour polymorphism. In light of this, Krüger and colleagues (2001) stated that Common buzzards would exhibit a rarely observed case of heterozygote advantage in the wild.

In the same study, the authors theoretical modelled different patterns of mate choice for the Common buzzards. The pattern of positive assortative mating they observed – i.e., non-random mating in which individuals with similar phenotypes mate with each other, best explained how fitness consequences could maintain genetic variation (Krüger et al. 2001). However, they also suggested that this mating pattern is maladaptive for the Common buzzards: to produce offspring with the highest fitness (i.e., the intermediate morph), light or dark individuals should mate with the opposite morph instead (disassortative mating).

Figure 1.2: Pictures representing the plumage coloration gradient in Common buzzards, divided in 7 morph types by Christiaan de Vries on the basis of body characteristics.

The mechanisms leading to fitness differences among morphs are still unclear. Krüger and colleagues tried to find several potential causes, such as parasite infection levels in nestling morphs (Chakarov et al. 2008), variation in aggressive behaviour of adult morphs (Boerner and Krüger 2009), differences in immunity (Chakarov et al. 2016), and correlations between fitness-related traits and heterozygosity (Boerner et al. 2013). In particular, Boerner and Krüger (2009) found that both intra-specific and inter-specific aggression differs between and within morphs, leading to a complex pattern on the population level, making it difficult to explain fitness differences among morphs. Chakarov et al. (2008) found that two parasite species in nestlings -*Carnus haemapterus* and *Leucocytozoon toddi*- may exert selection pressures in opposite directions on the melanism of their host, thus making intermediate buzzards better protected against endoparasites and not too attractive for ectoparasites. However, it was not possible to generalize this result for both sexes and in different foraging conditions, in turn making it difficult to explain fitness differences among morphs caused by parasite infections. Chakarov et al. (2016) explored the hypothesis that the differences in pigmentation corresponded to differences in immunity. However, no relation was found between the strength of immune responses and the melanisation gradient. These results indicate that there is most likely no simple correlation between immune responses and plumage morphs or between aggressive behaviour and plumage morphs, and that the latter may depend by a combination of factors.

Study population and study area

I investigated a breeding population of Common buzzards located in East-Friesland, The Netherlands (figure 1.3). This population has been intensively monitored since 1996 by two field ornithologists, Christiaan de Vries and Anneke Alberda. The number of nests checked during this time period remained largely stable (mean ±SD = 91 ±12, range 65-111), supported by a constant effort of this team to defend the population study from poaching. Since the establishment of the Dutch national working group for raptors (WRN) in 1982, the focus of the monitoring by the instigator Rob Bijlsma, by members Christiaan de Vries, Anneke Alberda and other ornithologists, has been to end the prosecution of birds of prey (Bijlsma 2007). Research about birds of prey is taking an increasing place within protection activities. Knowledge about behavioural and breeding biology thus proved to be indispensable (Bijlsma et al. 1994).

In addition to protecting Common buzzards from poaching, and in contrast to other monitored populations of Common buzzards, Christiaan de Vries also recorded the plumage coloration of individuals, adults and juveniles. This meticulous work made his dataset unique for evolutionary studies of colour polymorphism. The field ornithologist divided the plumage coloration gradient in 7 morph types on the basis of body characteristics (mainly front and underwing coverts, figure 1.2). This approach was adopted to more easily recognize individuals in the field. During each breeding season, the nests were checked several times by climbing the trees, and several reproductive parameters and biometric measurements were collected. During the rest of the year, a big effort was made to collect all moulting

feathers of adults and dispersing juveniles and to catch resident and floater birds, so to have as many individuals identified as possible.

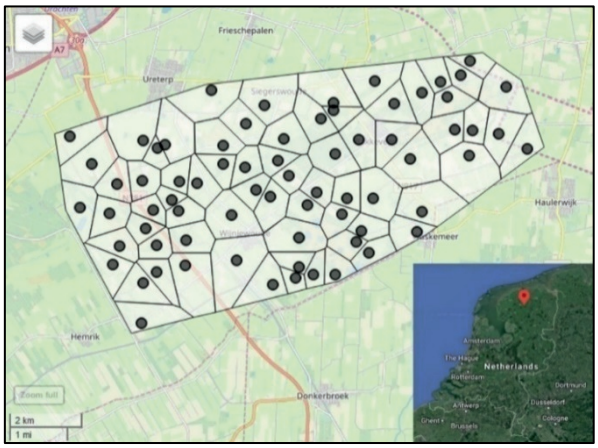

Figure 1.3: Map of the study area in year 2016. Dots represent nest positions. Panel on the right bottom shows where the area is (red marker) in The Netherlands.

Thesis focus

This PhD thesis is inspired by the incredible possibility to study an extremely interesting evolutionary topic thanks to the availability of a large and valuable data set. This thesis is based on long-term monitoring programs coordinated by Werkgroep Roofvogels Nederland (WRN) and builds upon enormous knowledge about biological trends of Common buzzard morphs in the last decades.

Since a major publication in 2001, the Common buzzard has become a species of interest in the study of the evolutionary ecology of colour polymorphisms. In fact, during the last decades, this raptor has been intensively studied in Germany (Krüger and Lindström 2001; Krüger et al. 2001; Chakarov et al. 2008, 2013; Boerner and Krüger 2009; Boerner et al. 2013). However, despite the species being very common and broadly distributed, it cannot be defined as a model species for colour polymorphism, as these studies are only restricted to this one German population.

Our objective was to replicate the study on fitness consequences associated with plumage colour morphs in the Common buzzard, but in a different environ-mental context. Replications in evolutionary ecology are generally relatively rare because they require long-term datasets collected in the wild to monitor the lifetime of individuals in a population (Nakagawa and Parker 2015). However, replication is necessary to validate findings and it is a basic requirement for the advancement of any field of research to be able to generalize. In nature many variables are beyond the control of researchers and studies cannot be perfectly replicated. Results in ecological and evolutionary studies often rely on specific ecological settings, that can yield different outcomes, emphasizing even more the importance of studying the ecological causes underlying selection.

This thesis will focus on Common buzzards from an intensively monitored Dutch population and aims to deepen our understanding of how plumage colour polymorphism can be maintained in this species. The main aims of my thesis were: (1) quantify more in detail how variable plumage coloration is in Common buzzards; (2) investigate how heritable plumage coloration is in our population and which inheritance system might it follows; (3) explore morph frequencies, and fitness differences among those morphs, in our population; (4) finally, describe patterns of natal dispersal in juvenile buzzard morphs.

Approach

To address the multidisciplinary aims of this thesis, we used a variety of techniques, from sophisticated data analyses to fieldwork including the deployment of GPS-transmitters on Common buzzards (figure 1.4). Thanks to the large long-term database, we were able to apply different analytical and statistical approaches. First, image analysis was performed on photographs taken in the field to first describe coloration patterns. Then, an animal model approach --a type of mixed-effects model using known genetic relationships between individuals-- was used to look at the quantitative genetics of plumage coloration. Finally, to estimate survival of individuals we used capture-recapture data with the program MARK and combined buzzard temporal data with vole counts and climatic data for The Netherlands. When moving to the spatial dimension of plumage colour polymorphism, we followed movements of dispersing Common buzzards by means of telemetry using 19-25 g solar-powered GPS-GSM transmitters. Tracking data were also combined with data on land cover (CORINE) to investigate habitat use by the morphs.

Figure 1.4: Common buzzard juvenile with solar panel GPS-GSM transmitter.

Outline of the thesis

Understanding how plumage colour polymorphism is maintained in Common buzzards requires a lot of basic knowledge about the colour polymorphism itself, in addition to the ecology and behaviour at the breeding site of adult Common buzzards and movements of juveniles before reproductive maturity. In **chapter 2**, I described the type of polymorphism present in the Common buzzards. I looked at Common buzzards colour variation qualitatively and quantitatively and tried to establish whether the polymorphism in this species is best quantified as a discrete or continuous trait. Hence, I made use of digital photographic material and used pixel coloration to quantify variation. To be able to compare our results to published literature, I also matched scoring systems between ours and previous studies. Lastly, I investigated whether an individual's plumage pattern is invariant through life by scoring morphs of individuals that were photographed for multiple years.
In **chapter 3,** I explored plumage inheritance patterns. Using social pedigree data from the wild, with juvenile birds with known parental morphs, me and my colleagues confirmed the hypothesized genetic basis of the trait, and explored whether this follows the earlier proposed Mendelian inheritance patterns. In **chapter 4**, I looked at fitness consequences and mate choice patterns of plumage trait variation, to better understand the maintenance of this polymorphism over evolutionary time. I examined the correlations between morph and adult apparent survival, breeding success, annual number of fledglings produced and cumulative reproductive success. Moreover, I show temporal variation in morph frequencies with 20 years of breeding data. After looking at adults in their breeding territories, I change scenario. In **chapter 5**, I looked at spatial and behavioural variation in plumage coloration for juvenile buzzards dispersing from their natal sites. I correlated the movement data collected by GPS to natal dispersal for different morphs. I inspected movements in relation to explorative behaviour and to habitat choice. This relatively unknown phase in the life cycle likely is important for selection, as most of the mortality happens while young birds are searching for a future territory. At last, in **chapter 6**, I summarize the results of this thesis and place them in a broader context. I discuss what we have learned about Common buzzard colour polymorphism as a model study to understand the maintenance of polymorphisms in nature. Moreover, I discuss the future path of research to further improve our knowledge on colour polymorphism in this species from different perspectives.

2

Classification and temporal stability of plumage variation in Common buzzards

Elena Frederika Kappers
Nayden Chakarov
Oliver Krüger
Anna-Katharina Mueller
Mihai Valcu
Bart Kempenaers
Christiaan Both

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Abstract

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.

Introduction

Investigations into the adaptive functions of animal coloration are widespread in behavioural and evolutionary biology. Studies of 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 and Blouin 2000; Ferguson-Lees and 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 and Krüger 2003; Hugall and 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 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, 2016), studying buzzards in Germany, refer to Blotzheim and Bauer (1997) 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, incl. 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 2004b), 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 investigating the genetic inheritance of the colour morph of both parents and their offspring just before they fledge.

Materials and methods

Image analysis

To quantify plumage colour variation, we carried out an image analysis using photographs of Common 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 and 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 (version 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 et al. 2015) 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 2.1).

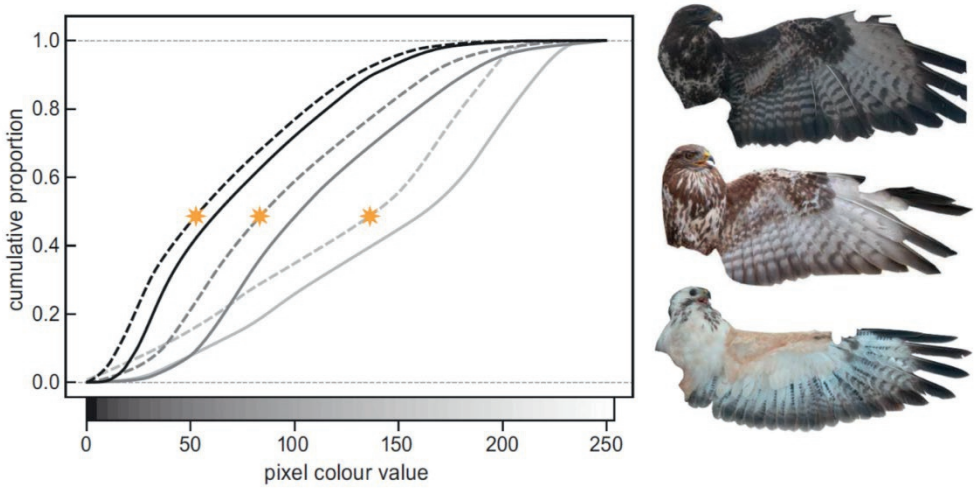

Figure 2.1: Cumulative frequency distribution of the pixel values (0=black, 255=white) of original and standardized photographs (solid and dashed lines respectively) of three different morph types. Yellow stars refer to the standardized median pixel value for each bird on the right. Black curves: upper 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).

First, we tested for multimodality in the frequency distribution of the colour index of the 93 individuals using Hartigans' dip test for unimodality (Maechler and Ringach 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 quantitative colour index and one with the qualitative morphs as 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 2.2.

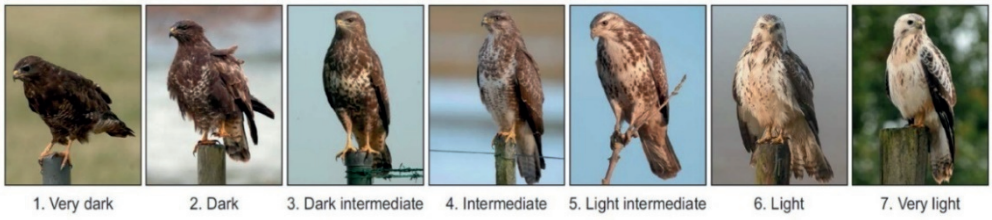

Figure 2.2: Example photos illustrating the seven-morph categorization from very dark to very light. (1) Very dark: 0-2% light plumage on the chin, throat, breast and flanks (hereafter front) and underwing coverts. (2) Dark: individuals are overall dark with a little white, having 3-10% light plumage 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 morph: 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.

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.

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 2.1, figure 2.3). 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.

ID	Age	Date		% Light plumage				Morph	
				Front		Underwing			
	1st photo	1st photo	2nd photo	1st photo	2nd photo	1st photo	2nd photo	1st photo	2nd 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

Table 2.1: 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 ± SD.

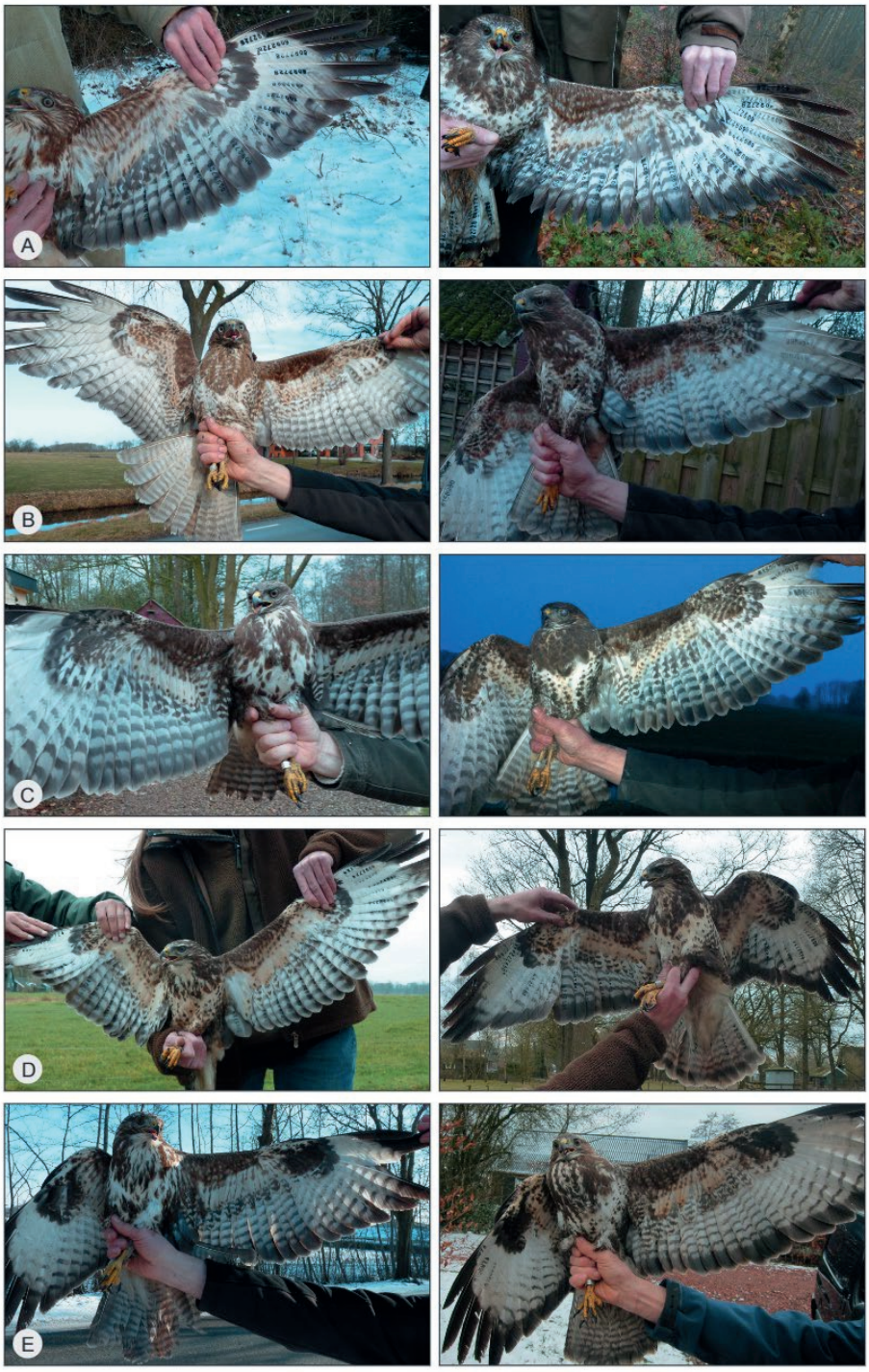

Figure 2.3: Paired photographs of 10 individual Common buzzards from Friesland, The Netherlands, taken first at juvenile age (left pictures) and after at least one moult cycle (right pictures). Letters refer to buzzard ID shown in table 2.1.

Figure 2.3: Continued.

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 2.4). 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).

Median pixel values correlated strongly with scores in the seven-scale morph categories ($r = 0.70$, $P < 0.01$; figure 2.5). Plumage colour, both when expressed quantitatively or qualitatively, did not differ between individuals of different age or between males and females (figure 2.6, table 2.2).

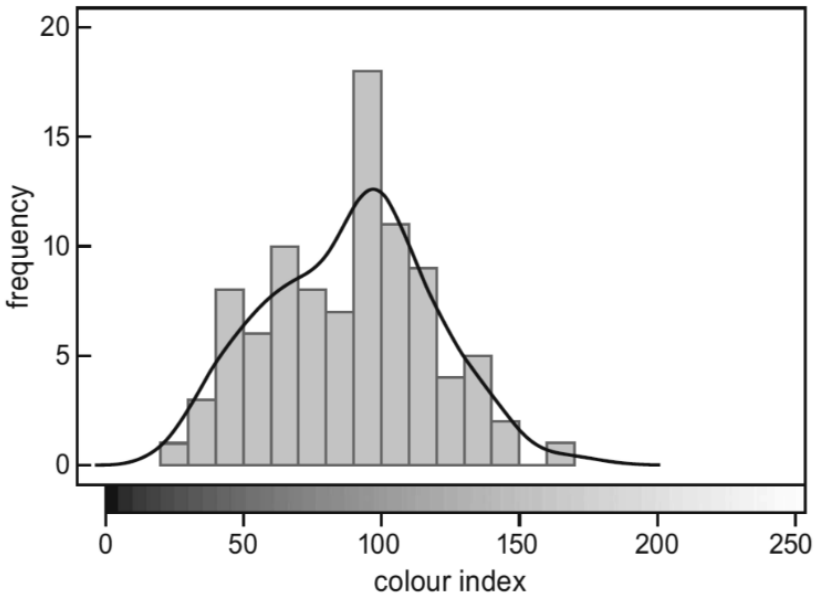

Figure 2.4: Frequency distribution of non-transformed median pixel values as colour index (0=black, 255=white) for 93 individual Common buzzards based on photographs of the front and underwing. The superimposed curve represents the frequency density distribution.

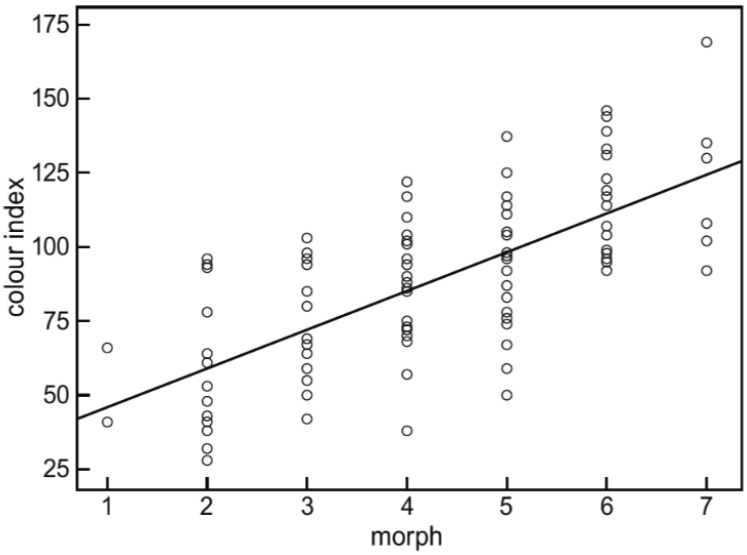

Figure 2.5: Relation between quantitative measurement and qualitative score of plumage coloration for 93 Common buzzards. The line depicts the regression line (r=0.70, P<0.01). The colour index varies between black (0) and white (255). The morph score varies between very dark (1) and very light (7).

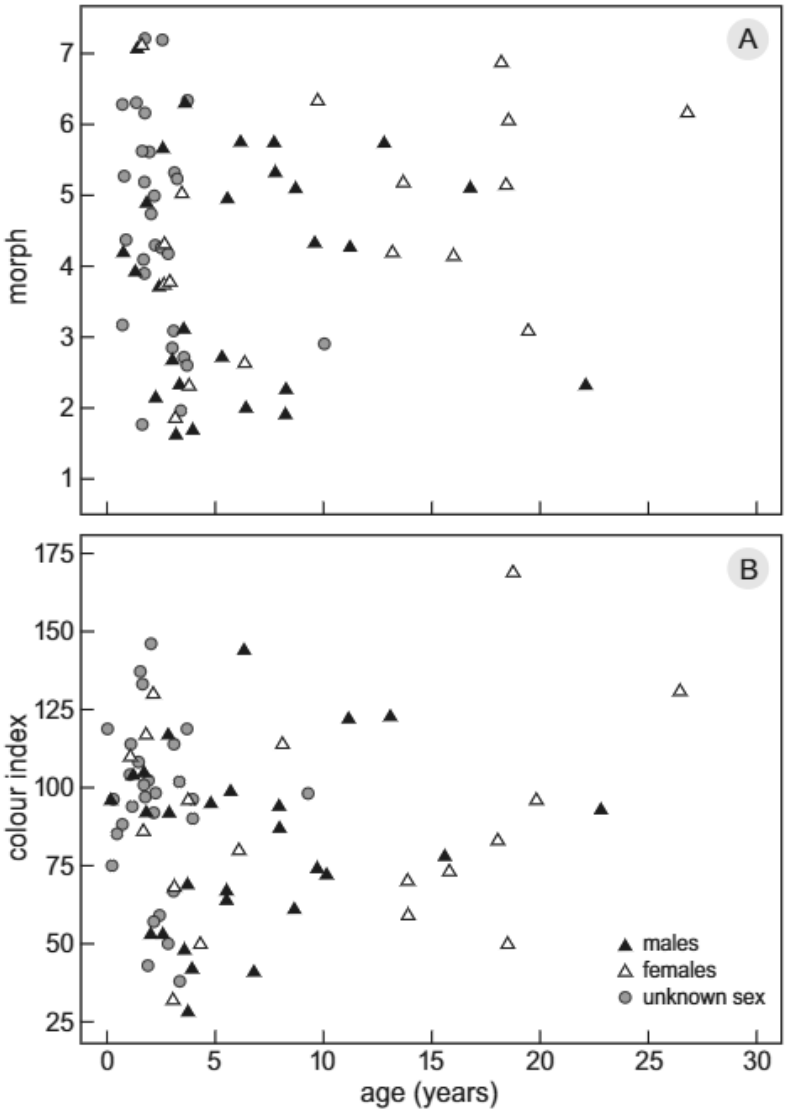

Figure 2.6: Plumage colour of Common buzzards expressed (**A**) qualitatively as morph score (1=very dark, 7=very light) and (**B**) quantitatively as median pixel value in relation to age for 74 individuals of known age (filled triangles: males, open triangles: females; grey circles: unknown sex; data points were jittered to avoid overlapping). Plumage colour was unrelated to age (see table 2.2 for statistical details).

Table 2.2: 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.
[1]Estimate for males relative to females (=0).

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 2.7). 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 2.7).

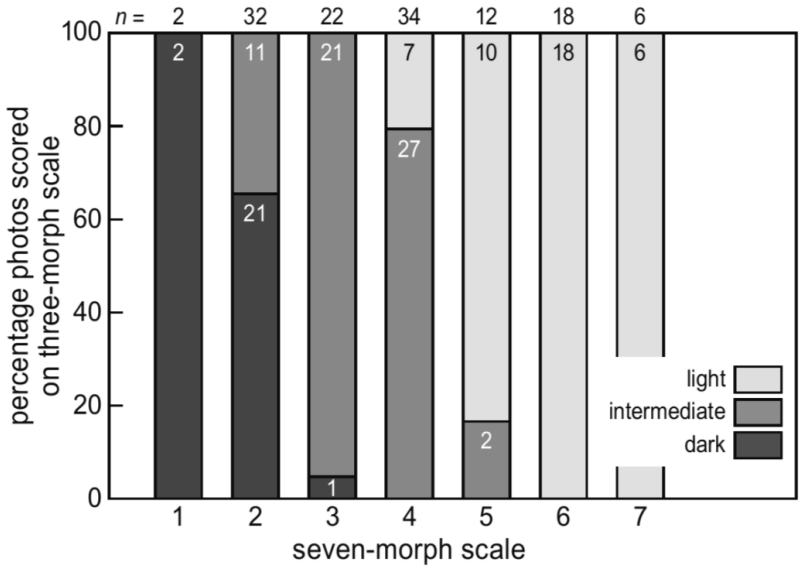

Figure 2.7: Comparison of two morph-scoring systems for the categorization of 126 Common buzzard photos. The Y-axis represents the percentage of photos scored dark (black), intermediate (dark grey) or light (light grey) on the three-morph scale. The X-axis gives the morph on the seven-morph scale from very dark (1) to very light (7). *n* refers to the number of individuals (photos).

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±0.108 (±SE); underwing: r = 0.798±0.096) or when comparing the morph categories (table 2.1). However, the estimated percentage of light plumage decreased significantly with age (on average 6.1% less 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 2.3; figure 2.8). 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 2.1).

Variable	Estimate ± SE	*z*-value	*P*-value
Intercept	63.87 ± 7.3		
Age	-6.13 ± 0.9	6.78	<0.001
Body part[1]	1.45 ± 2.3	0.62	0.89
Age × Body part[1]	3.01 ± 1.2	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.
[1] Categorical variable (front/underwing).
Front is reference category (=0).
Observer (*n*=13) and buzzard (*n*=10) identity were included in the model as random effects.

Table 2.3: 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.

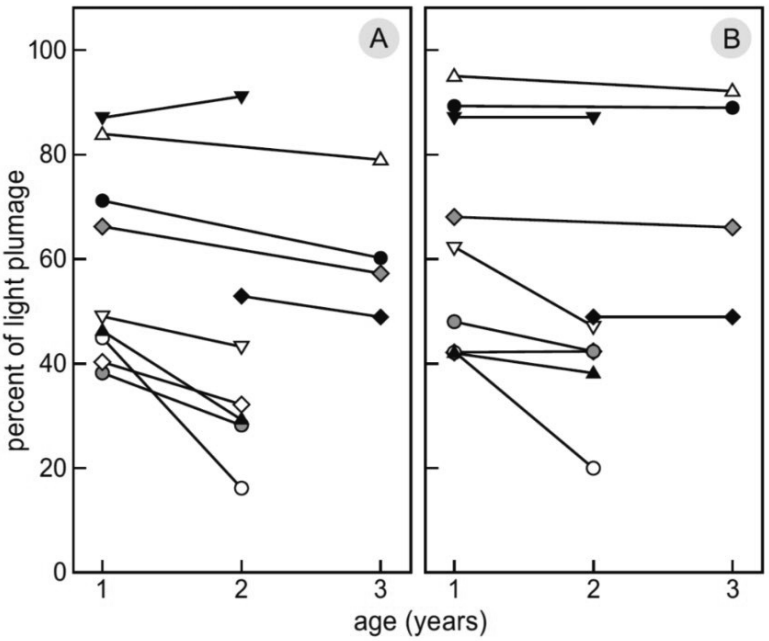

Figure 2.8: Changes in the percentage of light plumage with age for the front (**A**) and the underwing (**B**) in 10 Common buzzards from Friesland, The Netherlands. Each data point represents the mean score from 13 observers. Each individual is shown by a different symbol. Individuals became significantly darker with age, and this effect was stronger for the front than for the underwing (see table 2.3 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 and 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 (Preston 1980; Schmutz and Schmutz 1981; Palmer 1988; Briggs et al. 2011). Our quantitative analysis of plumage coloration00 showed that Common buzzards also express a continuous gradient with no distinctive multimodality (figure 2.4). 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 and Krüger 2009; Chakarov et al. 2015, 2016) aligns well with the underlying variation in the major gene. To test assumptions about the underlying genetic mechanism, it is important that models with different inheritance models are compared, as was e.g. done 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 2.7). 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 2.7). 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 (Antoniazza et al. 2010; Amar et al. 2014), 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 2.8). 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 and 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.

Acknowledgements

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Supplemental Material

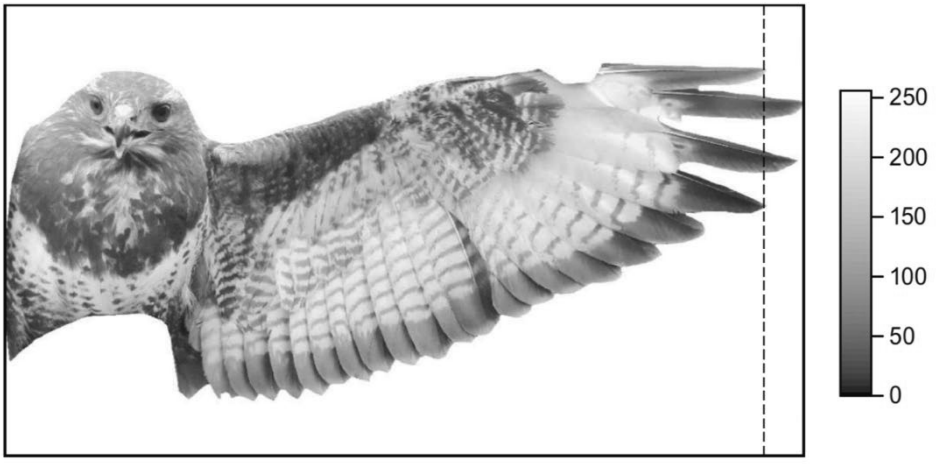

Figure S1: Example of one of the 93 photos being grey-scaled (0=black, 255=white) and rotated before image analysis. The vertical dashed line marks the right 5% of the photo length that was used to define the reference black colour.

3

Inheritance patterns of plumage coloration in Common buzzards *Buteo buteo* do not support a one-locus two-allele model

Elena Frederika Kappers
Christiaan de Vries
Anneke Alberda
Wolfgang Forstmeier
Christiaan Both
Bart Kempenaers

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Abstract

Balancing selection is a major mechanism to maintain colour polymorphisms over evolutionary time. In Common buzzards, variation in plumage colour was reportedly maintained by a heterozygote advantage: heterozygote intermediates had higher fitness than homozygote light and dark morphs. Here, we challenge one of the basic premises of the heterozygote advantage hypothesis, by testing whether plumage colour variation in Common buzzards follows a one-locus two-allele inheritance model. Using a long-term population study with 202 families, we show that colour variation in buzzards is highly heritable. However, we find no support for a simple Mendelian one-locus two-allele model of inheritance. Our results rather suggest that buzzard plumage colour should be considered a quantitative polygenic trait. As a consequence, it is unlikely that the proposed heterozygote advantage is the mechanism that maintains this genetic variation. We hypothesize that plumage colour effects on fitness might depend on the environment, but this remains to be tested.

Introduction

One of the big questions in biology is how genetic variation is maintained in populations over evolutionary time. Some proposed mechanisms involve balancing selection with a form of frequency-dependent feedback, resulting in fitness benefits to the rare allele (Sinervo and Calsbeek 2006). Another form of balancing selection is overdominance, where heterozygotes have higher fitness than both homo-zygotes, but relatively few examples exist in natural populations (Allison 1964; Knief et al. 2017).

A prime example of suggested overdominance in nature concerns the colour polymorphism observed in Common buzzards *Buteo buteo* (Krüger et al. 2001). Colour polymorphisms are relatively common in raptors (Schmutz and Schmutz 1981; Briggs and Woodbridge 2010; Karell et al. 2011; Amar et al. 2013) and typically involve variation in the amount of melanisation. Some evidence suggests that this trait variation is determined by simple Mendelian inheritance (Roulin 2004b).

Common buzzard plumage varies along a light-dark continuum, but has been categorized into three morphs (Kappers et al. 2017): light, intermediate and dark. Parent-offspring resemblance was consistent with a one-locus two-allele model, whereby intermediates (supposedly the heterozygotes) had higher fitness than light and dark morphs (supposedly the homozygotes; (Krüger et al. 2001)). However, this conclusion of simple Mendelian inheritance with a one-locus two-allele model was based on sparse data: overall 162 offspring with n<5 offspring for half of the parental combinations (Krüger et al. 2001). In two other Buteonine raptors, Ferruginous Hawks *B. regalis* and Swainson's Hawks *B. swainsoni*, similar patterns of inheritance have been suggested (Schmutz and Schmutz 1981; Briggs and Woodbridge 2010), but no heterozygote advantage was found in Swainson's Hawks (Briggs et al. 2011). However, also in these studies inheritance patterns were derived from exiguous sample sizes (n=5 offspring for 1 of the 3 possible parental combinations in (Schmutz and Schmutz 1981); n< 8 offspring for 3 of the 4 parental combinations in (Briggs and Woodbridge 2010)).

Our study aims to re-examine the hypothesis that morph variation in Common buzzards can be explained by a one-locus two-allele model. We tested whether the proportions of offspring of the different morphs produced by parents of known morph followed the predicted frequencies of a simple Mendelian trait. As an alternative, we examined whether the observed variation can be explained assuming polygenic inheritance with more continuous trait variation. To this end, we used our pedigree to calculate the heritability of plumage colour (i.e. the proportion of phenotypic variance explained by additive genetic variance), using a seven-morph plumage scale that better captures continuous variation (Kappers et al. 2017).

Materials and methods

Study population, colour score and pedigree information

Data on Common buzzards come from a long-term population study in Friesland, The Netherlands, started in 1996 (see appendix 1). Since 2001, all breeding Common buzzards and their 18-53 day old offspring (mean 33.8 days ± 3.6 SD) were colour-scored by one observer (CdV), using a seven-morph scale ranging from very dark to very light (Kappers et al. 2017). Juvenile plumage colour does not change substantially later in life (repeatability: r>0.74; Kappers et al. 2017).

We assembled a two-generation pedigree of 1279 birds, including 989 juveniles scored as fledglings between 2001 and 2016, and their 292 parents. The pedigree was based on field observations (i.e. direct sightings, photographs, captures, and identification based on moulting feathers), assuming strict monogamy. There is no evidence for intraspecific brood parasitism in buzzards and extra-pair paternity (EPP) is presumably rare. EPP levels reported in other socially monogamous raptors are low (for a review see table 1 in (Roulin et al. 2004)) and in a related *Buteo* species, 5% of the offspring were extra-pair (Briggs and Collopy 2012). Previous work showed that extra-pair paternity has a negligible impact on quantitative genetic estimates if the EPP level is low (<20% of offspring) and if sample sizes are sufficiently large (Charmantier and Réale 2005). Fathers produced on average 6.7 (median 5; range 1–31) and mothers 6.5 (median 4; range 1–31) offspring during the study period. In total, 976 mother-offspring relationships, 978 father-offspring relationships, 4157 full-sibling links and 10869 half-sibling links were informative for the heritability analysis. Pedigree statistics were performed using the R package pedantics (Morrissey and Wilson 2010).

Inheritance pattern of colour morph

To examine the one-locus two-allele model of inheritance, we repeated the analysis presented in (Krüger et al. 2001). First, we converted our seven-morph scheme into the three-morph scheme (light, dark, intermediate) that best approached the previous classification (see (Kappers et al. 2017)). As scoring schemes could not be perfectly matched, we examined four alternative scenarios of lumping individuals into the three-morph scheme (see appendix 2). The expected offspring morph frequencies were solely based on the phenotypes of both parents (see table 3.1). We used a Pearson's chi-square exact test on counts in StatXact (v. 4) to compare observed frequencies between parental combinations or between studies.

Heritability of plumage colour

We estimated the heritability of plumage colour (using the seven morphs) with quantitative genetic methods, assuming continuous variation. We constructed a linear mixed effect model incorporating relatedness information ("animal model", Kruuk 2004) to

partition phenotypic variance into autosomal additive genetic variance and environmental variance. As random effects, we included birth-year (to account for annual fluctuations in environmental conditions), nest (to account for shared natal environment), and mother and father identity. In all analyses, we combined data from female and male offspring and we initially included offspring sex as fixed effect. Because this effect was not significant, we excluded it in the final models. We fitted the animal model using a Bayesian framework implemented in R (version 3.3, R Core Team 2016) with the package MCMCglmm (Hadfield 2010). We chose weakly informative priors (inverse-Gamma distribution with nu=0.002 and V=1). Models were sampled every 10 iterations, with an initial burn-in of 100,000, for 1,000,000 samples, which resulted in autocorrelation <0.05 for all parameters. Posterior means and 95% credible intervals were estimated across the thinned samples for the mean effect and variance ratios.

Results and discussion

In contrast to conclusions from a previous study on Common buzzard morphs (Krüger et al. 2001), we found no support for the one-locus two-allele model of inheritance (table 3.1). Across all scoring scenarios, the observed segregation deviated substantially from the expected one (table 3.1, figure S1 and table S1).

Most importantly, intermediate offspring were greatly overrepresented in Intermediate x Intermediate pairs and underrepresented in Dark x Light pairs. I x I pairs should produce fewer intermediates (expected: 50%) than D x L pairs (expected: 100%), but observed frequencies are significantly in the opposite direction (p<0.001).

		Observed morph (%)			**Expected morph (%)**		
Parents	**$N_{offspring}$**	**D**	**I**	**L**	**D**	**I**	**L**
D × D	97	83.5	**16.5**	0	100	0	0
D × I	350	47.1	48.3	**4.6**	50	50	0
D × L	32	**18.8**	43.8	**37.5**	0	100	0
I × I	258	15.1	**74**	10.9	25	50	25
I × L	138	**2.9**	31.9	**65.2**	0	50	50
L × L	94	**1.1**	**14.9**	84	0	0	100

Table 3.1: Inheritance of plumage colour morph in Common buzzards from Friesland, The Netherlands. Morph classes are dark (D), intermediate (I) and light (L) scored under scenario 1 (see appendix 2). Observed morph shows percentage of offspring of each parental combination. Expected morph is the percentage of offspring of each morph expected under a one-locus two-allele model with intermediates being heterozygote. $N_{offspring}$ indicates total number of offspring from each parental combination. Bold print highlights overrepresented categories.

To assess why our conclusions deviate from those presented earlier (Krüger et al. 2001), we compared sample sizes and observed offspring morph frequencies between the two studies (table S2). The observed frequencies are remarkably similar and do not differ significantly even when using anti-conservative tests on count data that ignore the non-independence of offspring from the same nest or pair (all $p \geq 0.14$ in table S2).

Using our seven-morph classification, the animal model (model 1 in table 3.2) gives a heritability estimate for plumage colour of h^2=0.82 (95% CrI: 0.75 – 0.88). Shared nest environment and birth-year effects did not explain additional variation and neither did they alter the estimates of heritability, nor the maternal or paternal effects (model 2 in table 3.2). The effect of mother identity was not larger than the effect of father identity (table 3.2; 95% CrI of $(V_M-V_F)/V_P$: -0.1 – 0.1), suggesting no or minimal additional maternal effects (e.g. via egg composition) on offspring plumage colour.

Model	$V_A / V_P = h^2$	V_M / V_P	V_F / V_P	V_N / V_P	V_Y / V_P	V_R / V_P
1	0.82 (0.75 -0.88)	0.06 (10^{-3} -0.11)	0.05 (10^{-3} -0.10)			0.08 (0.03 -0.13)
2	0.81 (0.75 -0.87)	0.06 (10^{-3} -0.11)	0.05 (10^{-3} -0.10)	0.006 (6×10^{-5} -0.02)	0.003 (10^{-3} -0.01)	0.07 (0.03 -0.12)

Table 3.2: Proportion of variances and their corresponding 95% CrI from animal models used to partition phenotypic variance (V_P=2.24) into autosomal additive genetic (V_A) and environmental components of variance (V_M=mother identity, V_F=father identity, V_N=nest, V_Y=birth year; V_R=residuals).

These results, combined with the observation that colour variation in our population is rather continuous and unimodal (Kappers et al. 2017), suggest that plumage colour in buzzards should be considered a quantitative polygenic trait. This is contrary to conclusions based on inheritance patterns of melanic coloration in most other bird species (Roulin 2004b), where the melanic forms can either be dominant (Cooke and Cooch 1968; Schmutz and Schmutz 1981; Karell et al. 2011) or recessive (O'Donald 1983; Amar et al. 2013) (but note that this includes species with two distinct morphs (Cooke and Cooch 1968; O'Donald 1983; Amar et al. 2013) as well as species with a more continuous colour variation (Schmutz and Schmutz 1981; Karell et al. 2011).

In our buzzard population, plumage colour was highly heritable, independent of sex, and not influenced by environmental factors (table 3.2). Quantitative genetic studies of plumage coloration in birds such as Tawny Owls *Strix aluco* (Karell et al. 2011), Barn Owls *Tyto alba* (Roulin and Dijkstra 2003) and Common Kestrels *Falco tinnunculus* (Kim et al. 2013) showed similar high heritability values (h^2=0.80, 0.81 and 0.67-0.83 respectively). This implies that selection can act on the trait and that the variance is either selectively neutral or a mechanism exists that keeps the polymorphism stable.

The maintenance of the colour polymorphism in Common buzzards has previously been explained by heterozygote advantage (higher fitness of the intermediate morph), but

the present results question this explanation. Under a one-locus two-allele model, heterozygote advantage is sufficient to maintain a stable polymorphism where both alleles should be equally common in the population. However, in a polygenic inheritance system as supported by our data, overdominance would not be an effective mechanism for maintaining many alleles at individual loci (Kimura and Crow 1964) and it is more likely that variation is maintained through genotype-environment interactions (Gillespie and Turelli 1989). We suggest the testable hypothesis that the fitness effects of plumage colour are environment-dependent, which may explain geographic variation in morph frequencies (Gillespie and Turelli 1989).

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Ethics

Birds were handled by personnel with ringing license (VT 930).

Data accessibility

Data are available from the Open Science Framework (osf.io/3947z).

Supplemental Material

Appendix 1. Study site

The study site encompasses a 5724-ha area with 1400 ha of forested patches, centred at 53°04'09.2"N, 6°13'46.6"E, and contains on average 76±12 SD breeding pairs/year over a 20-year period.

Appendix 2. Colour morph scoring

To convert our seven-morph colour scoring scheme into the three basic morph types light, intermediate and dark, we used four different scenarios, based on (Kappers et al. 2017): (1) 1-2=dark, 3-4-5=intermediate, 6-7=light; (2) 1=dark, 2-3-4-5=intermediate, 6-7=light; (3) 1=dark, 2-3-4-5-6=intermediate, 7=light; (4) 1-2=dark, 3-4=intermediate, 5-6-7=light. The first scenario is represented in table 3.1 in the main text, and is based on the best fit when the authors of the original paper scored buzzard pictures that we also scored on our seven morph scale (see Kappers et al. 2017).

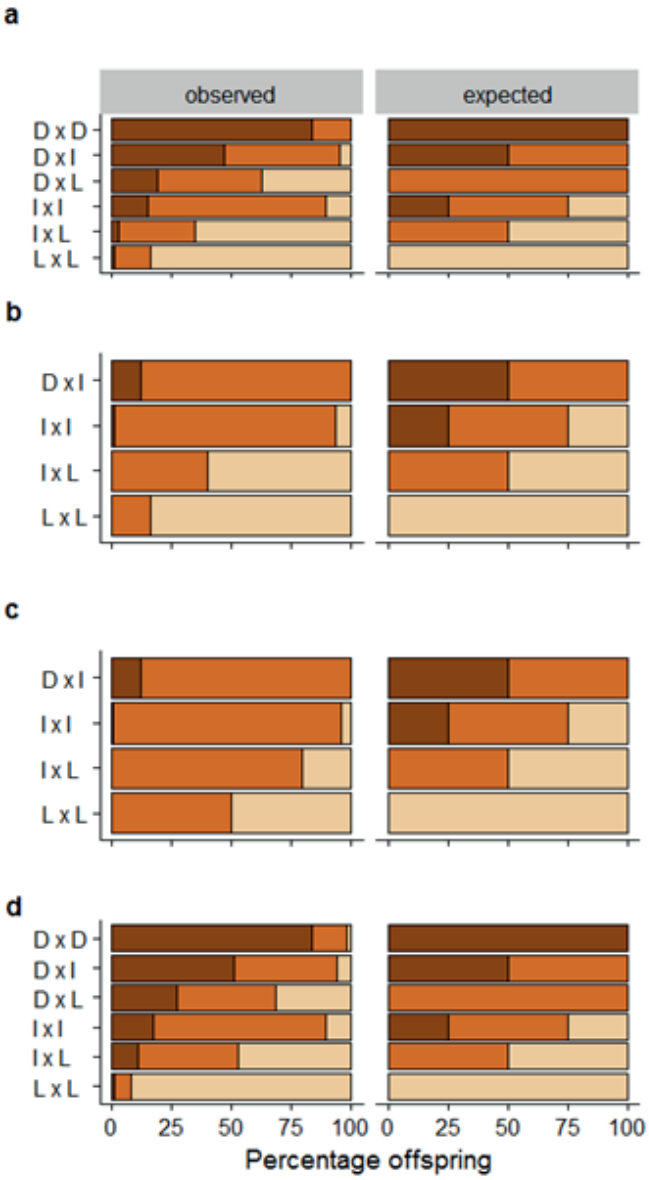

Figure S1: Observed and expected inheritance of plumage colour morph for all parental combinations in Common buzzards from Friesland, The Netherlands. (a)-(d) show the results for scenarios 1-4 (see above), respectively. Bars represent percentages of offspring of each morph class (brown = dark, orange = intermediate, beige = light) observed in our study (left panel) and expected from a one-locus two-allele inheritance pattern with intermediates as heterozygotes (right panel) for every parental combination shown on the y-axis. See table 3.1 and S1 for sample sizes and statistical analysis.

Table S1: Inheritance of plumage colour morph in Common buzzards from Friesland, The Netherlands. Morph classes are dark (D), intermediate (I) and light (L), scored under scenarios 2-4 (see above). Observed morph shows the percentage of offspring of each parental combination. Expected morph is the percentage of offspring of each morph expected under a one-locus two-allele inheritance pattern with intermediates being heterozygote. $N_{offspring}$ indicates the total number of offspring from each parental combination. Bold print highlights over-represented categories

		Observed morph (%)			Expected morph (%)		
Parents	**$N_{offspring}$**	**D**	**I**	**L**	**D**	**I**	**L**
Scenario 2							
D x I	34	11.8	**88.2**	0	50	50	0
I x I	671	0.9	**92.5**	6.6	25	50	25
I x L	170	0	40	**60**	0	50	50
L x L	94	0	**16**	84	0	0	100
Scenario 3							
D x I	34	11.8	**88.2**	0	50	50	0
I x I	865	0.7	**95.4**	3.9	25	50	25
I x L	64	0	**79.7**	20.3	0	50	50
L x L	6	0	**50**	50	0	0	100
Scenario 4							
D x D	97	83.5	**14.4**	2.1	100	0	0
D x I	283	50.9	43.1	**6**	50	50	0
D x L	99	**27.3**	41.4	**31.3**	0	100	0
I x I	159	17	**72.3**	10.7	25	50	25
I x L	136	**11**	41.9	47.1	0	50	50
L x L	195	**1**	**7.2**	91.8	0	0	100

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Table S2: Observed inheritance of plumage colour morph in Common buzzards from Friesland, The Netherlands (our study), and from a previous study in Eastern Westphalia, Germany (Krüger et al. 2001). Morph classes are dark (D), intermediate (I) and light (L), scored based on scenario 1 (see above). Observed morph shows the percentage of offspring of each parental combination. $N_{offspring}$ indicates the total number of offspring from each parental combination. P-values are based on Pearson's chi-square exact test performed on counts for 2 x 3 tables in StatXact 4.0.

	Our study				Previous study (Krüger et al. 2001)				
		Observed morph (%)				Observed morph (%)			
Parents	**$N_{offspring}$**	**D**	**I**	**L**	**$N_{offspring}$**	**D**	**I**	**L**	***p*-value**
D x D	97	83.5	16.5	0	2	100	0	0	1
D x I	350	47.1	48.3	4.6	22	36.4	64.6	0	0.25
D x L	32	18.8	43.8	37.5	4	0	100	0	0.15
I x I	258	15.1	74	10.9	90	22.2	64.4	13.3	0.21
I x L	138	2.9	31.9	65.2	41	2.4	48.8	48.8	0.14
L x L	94	1.1	14.9	84	3	0	0	100	1

4

Directional change of morph frequencies over time in a Dutch population of Common buzzards *Buteo buteo*

Elena Frederika Kappers
Christiaan de Vries
Anneke Alberda
Sylvia Kuhn
Mihai Valcu
Bart Kempenaers
Christiaan Both

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Abstract

We take advantage of 20 years of life history data collected in a Dutch population of Common buzzards *Buteo buteo* to replicate earlier studies on fitness consequences of colour polymorphism in this species. We examined morph differences in adult apparent survival, breeding success, annual number of fledglings produced and cumulative reproductive success. We found that fitness (cumulative reproductive success) differed among morphs, with the intermediate morph having highest fitness. Assortative mating for colour morph was observed, and we found that assortative pairs were more likely to produce offspring than disassortative pairs, and their pair bonds lasted longer. Over our long-term study we found a phenotypic change with an increasing proportion of intermediate morphs. This apparent evolutionary change did not just arise from selection on individual phenotypes, but also from fitness benefits of assortative mating. We hypothesize that spatial variation in selection pressures on colour morphs could be a mechanism that maintains this genetic variation, but it remains to be tested if plumage colour effects on fitness depend on dispersal and habitat choice.

Introduction

Identifying the processes that maintain genetic variation in populations over time is fundamental to evolutionary biology, as evolutionary responses are often based on the standing genetic variation (Lewontin 1974). One wide-spread type of genetic variation in animals is colour polymorphism: within-population variation in appearance across individuals independent of age and sex (Huxley 1955). Understanding the persistence of genetically determined phenotypic polymorphisms requires measuring the covariation between the genetic part of the trait and fitness (Roulin 2004b).

Several mechanisms have been proposed to explain the maintenance of polymorphisms, and one of these is balancing selection. Frequency-dependent selection is a form of balancing selection where the rare allele has higher fitness than the more common alleles (Smith 1982; Sinervo and Calsbeek 2006). Another rarely observed form of balancing selection is overdominance, where heterozygotes have higher fitness than both homozygotes (Allison 1964; Knief et al. 2017).

In birds, 3.5% of all species show plumage colour polymorphisms which are often genetically determined (Galeotti et al. 2003). Colour polymorphisms are particularly common in raptors (30% of species; Fowlie and Krüger 2003; Hugall and Stuart-Fox 2012). Among raptors, the Common buzzard, *Buteo buteo*, is one of the most variable species in terms of plumage colour. Individuals vary along a dark–light continuum, but for practical reasons this variation has often been categorized into three morphs: dark, intermediate and light (Kappers et al. 2017). Using parent-offspring comparisons, we previously showed that plumage colour is highly heritable (82%), independent of sex and not influenced by environmental factors such as shared nest environment and female and male parent identity (Kappers et al. 2018). Because the variation in these morphs has such a strong genetic basis, the covariation between phenotype and fitness can be considered as direct selection on the genetic component of the polymorphism. Previous studies on a German population provided evidence that the polymorphism could be explained by a one-locus two-allele inheritance system, with the intermediate morph being the heterozygote (Krüger et al. 2001): both lifetime reproductive success and adult survival were higher for the intermediate morph than for the light and dark morphs (see also Jonker et al. 2014). The authors concluded that heterozygote advantage maintains the colour polymorphism in this species (Krüger et al. 2001).

Fitness differences among morphs were also investigated in the related Swainson's hawk, *Buteo swainsoni* (Briggs et al. 2011). Similar to the Common buzzard, this species also shows continuous colour variation, which has been categorized in three morphs. Using 32 years of breeding data, Briggs, Collopy & Woodbridge (2011) found no evidence that intermediate individuals (presumed heterozygotes) had higher fitness; there were no differences in any of the examined fitness components between the morphs. Therefore, Briggs, Collopy & Woodbridge (2011) excluded both frequency-dependent selection and heterozygote advantage as mechanisms maintaining the colour polymorphism in this species.

Our aim is to replicate the study on fitness consequences associated with plumage colour morphs in the Common buzzard. Replications of this type are relatively rare (Nakagawa and Parker 2015), especially when carried out in the wild, because they require long-term datasets monitoring the lifetime of individuals in a population. Replication is essential to validate findings and it is a basic requirement for the advancement of any field of research to be able to generalize. Because outcomes in ecological and evolutionary studies often rely on specific ecological settings, it is likely that such replications will yield different outcomes, thereby showing the importance of studying the ecological causes underlying selection. An additional reason to replicate the previous study is that we recently showed that the colour polymorphism of the buzzard does not fit the originally proposed one-locus two-allele system of inheritance, but rather should be considered as a polygenic quantitative trait with high heritability (Kappers et al. 2018).

Here, we use a 20-year study of a population of Common buzzards from The Netherlands to replicate the empirical findings on fitness consequences of colour polymorphisms from the original publications (Krüger et al. 2001; Jonker et al. 2014). Specifically, we investigate differences among morphs in adult survival, annual reproductive rates and cumulative reproductive success.

An intriguing conclusion from the original study was that buzzard mate choice was maladaptive (Krüger et al. 2001), because pairs showed assortative rather than disassortative mating. This was supposedly maladaptive because to produce offspring with the highest fitness (the intermediate morph), light or dark individuals should mate with the opposite morph. Therefore, we also describe the mating patterns in relation to morph in our population and the fitness consequences of different mating combinations.

Materials and methods

Study site and population

C.d.V. and A.A. studied Common buzzards from 1996 onwards in Friesland, The Netherlands (53°04' N, 6°13' E). The study site encompasses a 5724-ha area with 1400 ha of forested patches. The larger patches are spruce, pine and larch-dominated (~ 1000 ha), whereas the smaller patches (~ 400 ha) are oak-dominated. The study area contains on average 81 ± 14 SD breeding pairs/year (range: 57-110). In each year, all territories were visited in late winter to determine whether they were occupied by a breeding pair. Breeding performance of each pair was assessed by multiple observations made both from the ground before egg laying and from climbing to and checking the nest before and after hatching. Here, we use data from a 20-year period (1996-2015), during which all breeding buzzards were colour-scored for overall plumage, using a seven-morph scale ranging from very dark to very light (Kappers et al. 2017).

Individuals were identified based on plumage colour and pigmentation patterns scored from direct sightings in the field, photographs, captures (N=90), and – in most cases – from collected moulting feathers, combined with the location of the observation (266

adult females and 244 adult males). Each year, C.d.V. and A.A. tried to collect moulting feathers of all females during incubation around the nest and for all males in their territory after the breeding season. Individual identification was based on visual comparison of the highly diverse colour patterns with collected feathers from previous years. We confirmed this individual assignment through genetic profiling with microsatellites using DNA from the shafts of a subset of collected moulted feathers. Identification based on feather phenotype was correct for 99% of 199 analysed feathers (see appendix 1 in supplementary material).

For analyses, we grouped individuals into dark, intermediate and light morphs following a three-morph scheme that best approached the previous classification (see Kappers et al. 2017). This allowed a direct comparison with previous studies in Germany (Krüger et al. 2001, and see introduction).

As our interest is also in potential ecological drivers of selection on colour morphs, we considered two environmental covariates that may affect annual variation in fitness components. (1) The North Atlantic Oscillation (NAO) index for the months December through March, which is indicative of the severity of the winter: positive values are typically associated with wetter and milder weather over western Europe, while negative values indicate drier and colder weather (updated from Jones et al. 1997). (2) An annual vole index, determined by the sum of the number of common vole *Microtus arvalis* holes in western Drenthe (approximately 20 km south from our study site) that were re-opened 24 hours after closing them in 35 grassland plots of 1 x 1 m in March and August (Bijlsma 2016). Common voles vary strongly in abundance between years, and are the primary food source for Common buzzards. The NAO and vole index were only weakly correlated (figure S1).

Adult apparent survival

We used Cormack-Jolly-Seber (CJS) models to analyse whether survival of breeding adults observed between 1996 and 2015 was associated with morph. The dataset included 266 females and 244 males (155 dark, 253 intermediate and 102 light individuals). The CJS models separate the survival probability from the re-sighting probability using a maximum-likelihood approach. We analysed the sexes separately because high mate fidelity in buzzards increases the probability of observing a pair, such that male and female partners are non-independent observations. We constructed our models using the program MARK (White and Burnham 1999) with package RMARK (Laake 2013) in R (R Core Team 2016).

Our initial model for each sex included morph and year. First, we assessed the fit of these general models by performing goodness-of-fit (GOF) tests using the program RELEASE (Burnham 1987). The GOF of the CJS models (test 2 and 3) was satisfactory (males: χ^2=198.29, df=112, P<0.0001; females: χ^2=165.80, df= 112, P=0.0007). We found no indication of significant overdispersion (GOF test: $\hat{C}_{males}$=1.77, $\hat{C}_{females}$=1.48), but we corrected for the lack of fit of the model to the data by adjusting $\hat{C}$ from 1.0 to 1.77 for males and to 1.48 for females.

We estimated two parameters: apparent survival (ϕ) and encounter probability (p). We used a hierarchical modelling approach, retaining only the best-ranked models from the previous step - based on Aikaike's Information Criterion (AIC) - before considering a new

suite of covariates (Burnham and Anderson 2002). As p was considered a nuisance parameter, we modelled it first to obtain the best fit for resighting probability. We added the factors morph (m) and year (t) to account for potential differences in detectability between the three morphs as well as among the years. We compared models based on a combination of Δ^{AICc} and model complexity (number of parameters) following Burnham & Anderson (2002). Models that only added complexity to a simpler model but did not improve the fit (usually falling within 2 AICc-values) were not considered competitive (Arnold 2010). As there was no support for a difference between morphs in resighting probability, we only kept the model with year in subsequent analyses (see table S1).

To model survival (ϕ), we used a first set of models with morph and year and their interactions (m × t) as factors. We fitted all five possible models to the data. Subsequently, we added environmental variables that might affect survival probability. The new continuous variables included: the NAO-index, the vole-index and the average number of fledged chicks from the previous year as a measure of how stressful the breeding season had been, in addition to a number of biologically plausible interactions between these variables (see table S2 for full set of 24 models). Figure S2 shows yearly variation in the ecological variables for the period 1996-2015. We did not include minimal age (or breeding career length), because the age together with year severely reduces the degrees of freedom.

Morph-assortative mating

We assessed the level of morph-assortative mating by calculating the Pearson's correlation between the colour morphs of pair members (for this we encoded 1 as dark, 2 as intermediate and 3 as light). We did this for two datasets, once considering all unique pair combinations of known colour morph from the entire study period (N= 400), and second, considering all breeding pairs repeatedly for each year of the study (N= 1566). For all breeding pairs we assessed the level of morph-assortative mating across and within years. Most of the breeding attempts in multiple years were with the same partner (females: 68%, males: 63%). In the remaining cases, individuals had multiple mates during their stay in the population (females: 19%, 10%, 2% and 1% with 2, 3, 4 and 5 mates, respectively; males: 22%, 9%, 4%, 1% and 1% with 2-6 mates, respectively). The significance of the Pearson's correlation coefficients was tested using a resampling procedure. The values of the morph of all individuals were randomized N= 100,000 times (over the years when comparing unique pair combinations with all breeding pairs; within year when considering breeding pairs repeatedly) and for each randomization we calculated the correlation coefficient. The significance level of the actual correlation coefficient is then given by $(n*2)/N$, where n is the number of randomized values that are equal to or more extreme than the observed correlation.

We tested whether pairs with different degrees of assortment with respect to the plumage morph differed in the duration of their pair bond. For all unique pair combinations of known colour morph (n=400), the level of assortment by morph was defined in three categories: "2" for pairs where both members have the same morph (dark-dark, intermediate-intermediate, light-light), "1" for intermediate-dark or intermediate-light pairs

and "0" for dark-light pairs. We used a linear mixed model with pair duration in years (log10-transformed) as the response variable and with the degree of assortment (factor with 3 levels) as the independent variable. We included as random intercepts the first year of breeding (n=20, which also accounts for shorter pair durations in more recent years), female identity (n=264) and male identity (n=242). Results were back-transformed for illustrative purposes.

Measures of reproductive success

For each breeding season, we examined reproductive success of all territories where the productivity was known and the morph of both adults had been scored (n=1359). We defined yearly reproductive success as the number of fledglings produced in that year, which varied between zero and four (mean ±SD: 0.9 ±1.0, including all territories; 1.8 ±0.8, n=732, excluding unsuccessful nests and non-breeding pairs). Nestlings were considered fledged if their presence was recorded in the natal territory after the expected fledging date. For pairs that had two nesting attempts in the same breeding season we only considered the last nest as the first attempts were unsuccessful.

We modelled variation in yearly reproductive success with a GLMM using package lme4 (Bates et al. 2015) in R (version 3.3, R Core Team 2016) using a Poisson distribution, a log-link and a Laplace approximation. As explanatory variables, we added morph of both attendant adults, the degree of assortment by morph of the pair (factor with 3 levels, see above), and "disturbance" (factor with three levels: nest disturbed by humans, nest take-over attempt by Egyptian goose *Alopochen aegyptiaca*, no evidence of disturbance). We also added female identity (n=259), male identity (n=239) and year (n=20) as random intercepts.

Additionally, we calculated cumulative reproductive success for both males (n=244) and females (n=266), as the total number of fledglings produced during their presence in the population which ranged from 0 to 38 for both sexes (mean ± SD = 5.2 ± 6.4, for males; 4.7 ± 6.4 for females). For 60% of all individuals in the analysis, cumulative reproductive success (CRS) equals lifetime reproductive success (LRS) – assuming that individuals that were not observed during three consecutive breeding seasons had died – while for the remaining 40%, it reflects their fledgling production up to 2015. We used cumulative reproductive success to not exclude successful individuals that were still breeding in the last three years of the study (13% of the 206 individuals for which CRS does not equal LRS had been recorded for at least 15 years). We also calculated lifetime fledgling production for the subset of 161 females and 143 males that were supposed dead in 2015, because they were not observed in 2013-2015.

Cumulative reproductive success and LRS were modelled using a GLMM with a Poisson distribution, a log-link and a Laplace approximation. As explanatory variable we added the morph of each individual. We included the first year of the breeding career of an individual as random intercept, to account for between-cohort variation and for the fact that in more recent years some individuals were still alive. Moreover, we analysed CRS by adding to the previous model the number of breeding attempts as covariate. To avoid bias in the

cumulative fitness estimates due to detection rates <100%, we re-ran the analyses excluding individuals that were missing in the dataset for more than 2 years (34 of 510 adults, 6.6%).

Changes in morph frequencies over time

We examined temporal variation in morph frequencies for both males and females across the 20 years of the study. We used the data from all individuals for which the morph had been scored in each year ($n_{females}$=1453 individual-years; n_{males}=1428 individual-years). Because some birds featured in multiple years, leading to pseudo-replication, we also assessed changes in morph frequencies of all individuals in their first year of breeding only ($n_{females}$=266; n_{males}=244).

We fitted a generalized linear model for each of the three morphs, where the dependent variable is the proportion of all individuals of a given morph and the independent variables are sex and year. The models were fitted with a binomial error distribution corrected for under-dispersion (i.e. using the quasi-binomial family). The robustness of the models was evaluated using non-parametric bootstrap with function Boot in package car (Fox and Weisberg 2011).

Results

Adult apparent survival

Capture–recapture analysis showed no difference among the three morphs in the probability of resighting (p). P varied among years both in females and in males (table S1). Based on these models, mean annual resighting rates were 0.86 (95% confidence interval, CI=0.83-0.87) for males, and 0.87 (CI=0.85-0.89) for females.

For both sexes we found little support for morph-dependent survival rates, with the model without other factors included having most support (table 4.1). For males, there is some support (delta AICc <2) for a model that includes morph, but effects of morph are at best minor (figure 4.1). Based on overlapping confidence intervals of the best supported null model, males had similar survival (estimate=0.90, CI=0.88-0.91) as females (estimate=0.88, CI=0.86-0.90). Models that included ecological covariates (reflecting yearly variation in winter severity, food availability and the investment during the previous breeding season) were not better supported than the null model (table S3, figure S1).

Model	*n*	AICc	DeltaAICc	Weight	Deviance
Males					
φ(.) p(t)	20	1128.624	0.0	0.719	665.47
φ(m) p(t)	22	1130.507	1.87	0.280	663.22
φ(t) p(t)	38	1153.688	25.84	0.000	652.90
φ(t + m) p(t)	40	1155.713	27.13	0.000	650.68
φ(t x m) p(t)	76	1222.682	94.05	0.000	638.94
Females					
φ(.) p(t)	20	1942.118	0.0	0.881	1098.37
φ(m) p(t)	22	1945.135	3.01	0.119	1097.26
φ(t) p(t)	38	1956.208	14.09	0.000	1074.85
φ(t + m) p(t)	40	1959.051	16.93	0.000	1073.46
φ(t x m) p(t)	76	1990.324	48.20	0.000	1026.14

Table 4.1: Results of a capture-recapture analysis for data on breeding male and female Common buzzards between 1996 and 2015. Buzzards are categorized by their colour morph (dark, intermediate and light). The analysis separates between survival probabilities (ϕ) that can be either constant (.), morph dependent (m), year dependent (t), or both morph and year dependent (m x t), and recapture probabilities (p) that are year dependent (t). All five possible models are displayed in decreasing order of AICc-values (fit to the data). Shown are the number of parameters (n), the corrected Akaike information criterion (AICc), Delta AICc (the difference in AICc between the current model and the best model), the proportional support for the model (i.e. the AICc weight) and the deviance. Models are corrected for overdispersion ($\hat{C}$=1.77 for male and $\hat{C}$=1.48 for female buzzards).

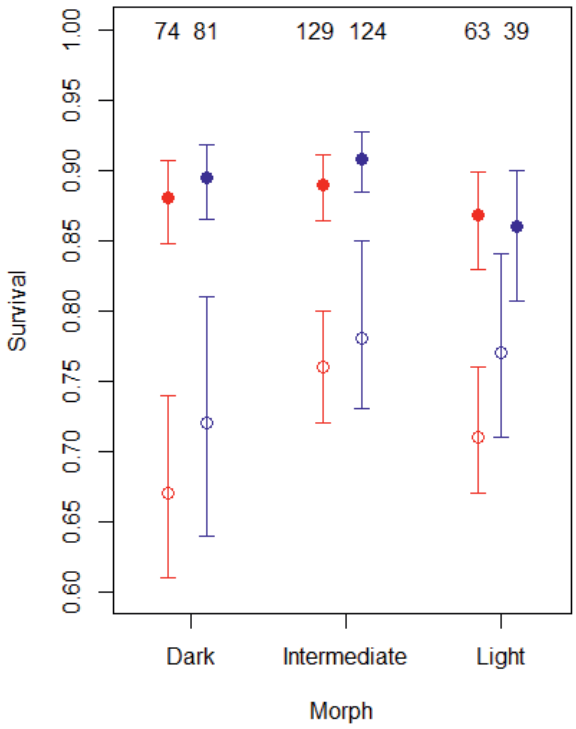

Figure 4.1: Apparent survival probabilities in relation to plumage colour morph for females (red) and males (blue). Filled dots show the survival estimates (± 95% CI) from our Dutch population, based on the capture-recapture model ϕ(m) p(t) (see Results and table 4.1). Open circles show the average survival probabilities (± se) for each morph in the German population after model averaging (data from Jonker et al. 2014: n_{males}=670, $n_{females}$=669). Numbers on top indicate sample sizes for our study.

Mating patterns

Common buzzards showed weak positive assortative mating with respect to colour morph (Pearson's r=0.13, n=400 unique pairs, *P*=0.01). The estimate of assortative mating was stronger when considering all breeding attempts (r=0.24, n=1566, *P*<0.001), suggesting that positively assorted pairs bred together for more years than disassortative pairs. Indeed, pair bond duration increased with the level of assortative mating (χ_2=16.459, *P*<0.001), where the disassortative pairs (scored as 0) had a significantly lower pair bond duration than pairs that were intermediately assorted (scored as 1) or highly assorted (scored as 2) (figure 4.2, table S4). Note that there was no difference in pair bond duration between light-light/dark-dark pairs (pooled, N = 64) and intermediate-intermediate pairs (N = 108; table S5).

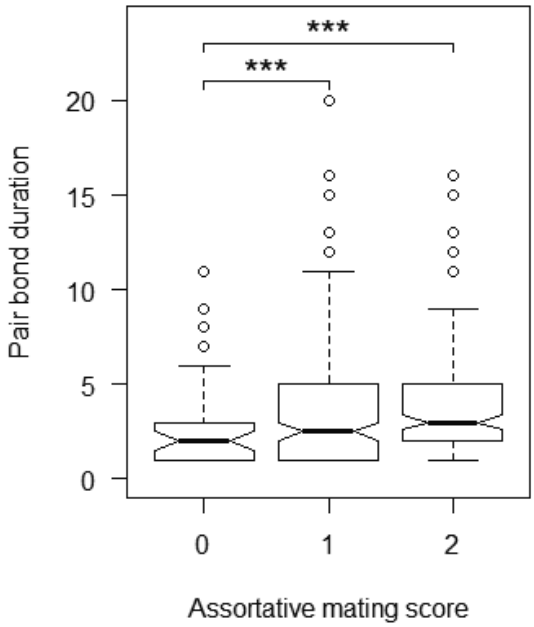

Figure 4.2: Pair bond duration in relation to the level of assortative mating based on plumage morph. Shown are boxplots of pair bond duration (the number of years a pair bred in the population) for three classes of assortment by morph: "0" for dark-light pairs (n=36), "1" for intermediate-dark or intermediate-light pairs (n=192), and "2" for pairs where both partners had the same morph (n=172). Circles indicate statistical outliers. Asterisks indicate P <0.001 (see table S4). Tukey post-hoc comparisons, 0-1: z=3.687, P<0.001; 0-2: z =4.016, P<0.001; 1-2: z =0.676, P=0.7.

We also estimated the level of assortative mating for each year separately. This showed a significant positive assortment in thirteen out of 20 years (all r> 0.18, all P<0.05 in 1999, 2001-04, 2006-07, 2009-13, 2015; figure S3). Interestingly, in the first three years of the study we found no support for positive assortative mating by morph, but levels increased and stabilized thereafter at annual correlation coefficients varying around 0.27.

Reproduction

Neither male, nor female morph explained any of the variation in the annual number of fledglings produced. However, pair assortment with respect to morph had a significant influence on reproductive success (table S6): assortative pairs fledged more offspring (figure 4.3a, b). This result was mainly driven by a difference in nest success (the probability that a brood produced at least one fledgling) among pairs with different degrees of assortment by morph (figure 4.3c, d; table S7).

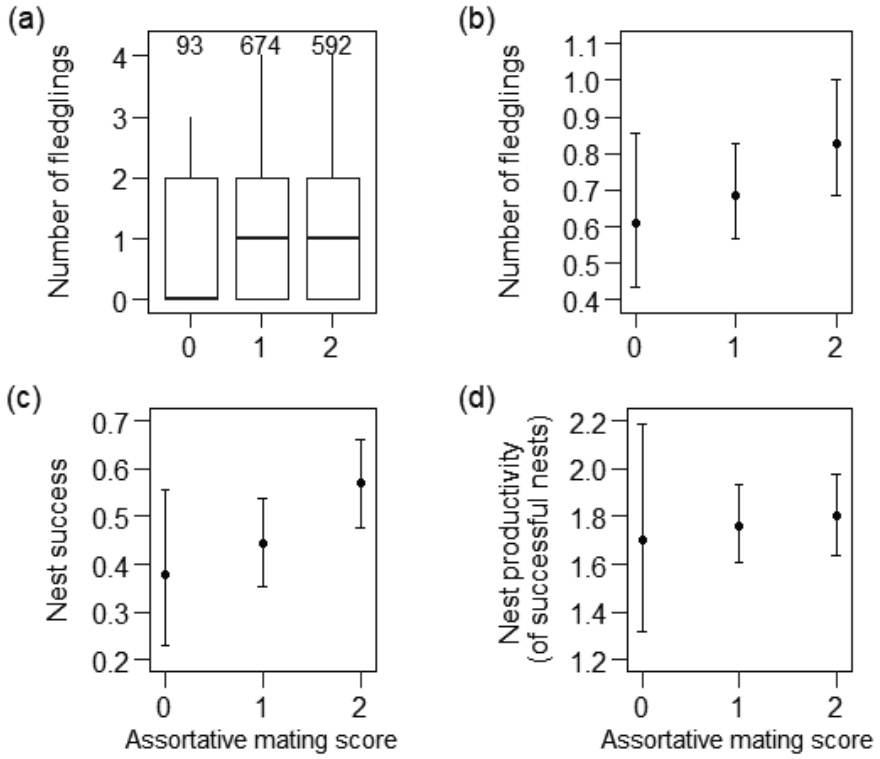

Figure 4.3: Yearly reproductive success in relation to the degree of pair assortment by morph. Three classes of assortment by morph are considered: "0" for dark-light pairs (n=93), "1" for intermediate-dark or intermediate-light pairs (n=674), and "2" for pairs where both partners had the same morph (n=592: n_{DxD}=156, n_{IxI}=324, n_{LxL}=112). (**a**) Boxplots of the raw data for the number of fledglings per brood (text on top represents total sample size for the three levels); (**b**) mean number of fledglings per brood ± 95% confidence intervals from the GLMM model with as independent variables female morph, male morph and disturbance: assortative pairs fledged more offspring (Tukey post-hoc comparisons, 0-1: z =0.70, P =0.75; 0-2: z =1.8, P =0.16; 1-2: z =2.33, P =0.047 (see table S6); (**c**) nest success, i.e. the probability of producing at least one fledgling (mean ± 95% CI, see table S7); (**d**) fledgling productivity when considering only successful nests (with at least one offspring fledged). Shown are means ± 95% CI (table S7).

Individual cumulative reproductive success was dependent on morph for both males (χ^2_2=6.04, *P*=0.05) and females (χ^2_2=13.361, *P*=0.001). In females, the intermediate morph had a significantly higher CRS than the dark morph and in males it had a significantly higher CRS than both extremes (figure 4.4, table S8). When re-running the analysis on the subset of individuals that were not missing for more than 2 years from the population, we found that the intermediate morph had significantly higher CRS than the dark morph in both sexes (table S9). Interestingly, when including the number of breeding attempts this was highly significant, while the effect of morph on CRS was no longer significant (table S10). When considering LRS on the subset of individuals that were assumed dead (after not having been observed in three consecutive breeding seasons) we found no significant effect of morph for females and a significant difference between intermediates and dark morphs for males (table S11).

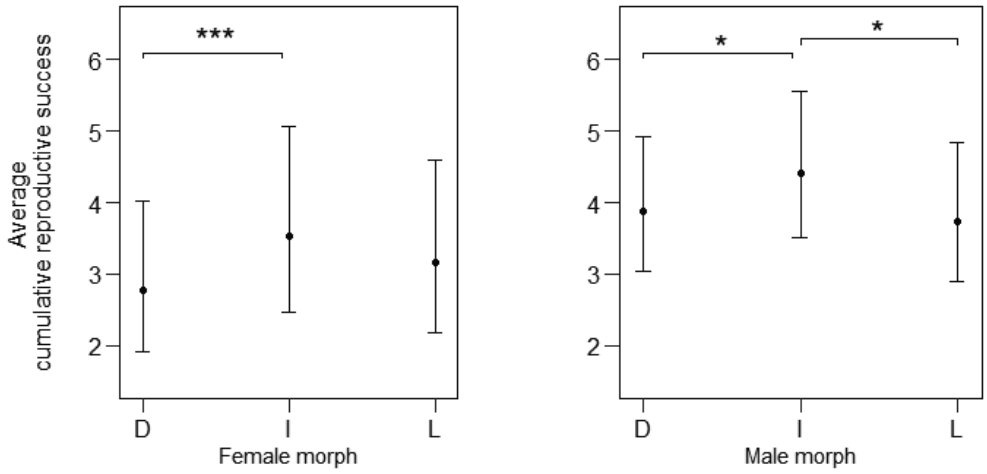

Figure 4.4: Cumulative reproductive success for females (n=266) and males (n=244), as the total number of fledglings produced during their presence in the population, in relation to the morph of each parent (D=dark, I=intermediate, L=light). CRS was modelled using a GLMM with a Poisson distribution, a log-link and a Laplace approximation (table S8). Shown are average cumulative reproductive success ± 95% confidence intervals.

Morph frequencies over time

Over the entire study period, 155 out of 510 individuals (30%) belonged to the dark morph, 253 (50%) belonged to the intermediate morph and 102 (20%) belonged to the light morph. Frequencies were similar for males and females: (males: 33% D, 51% I, 16% L; females: 28% D, 48% I, 24% L; numbers given in figure 4.5).

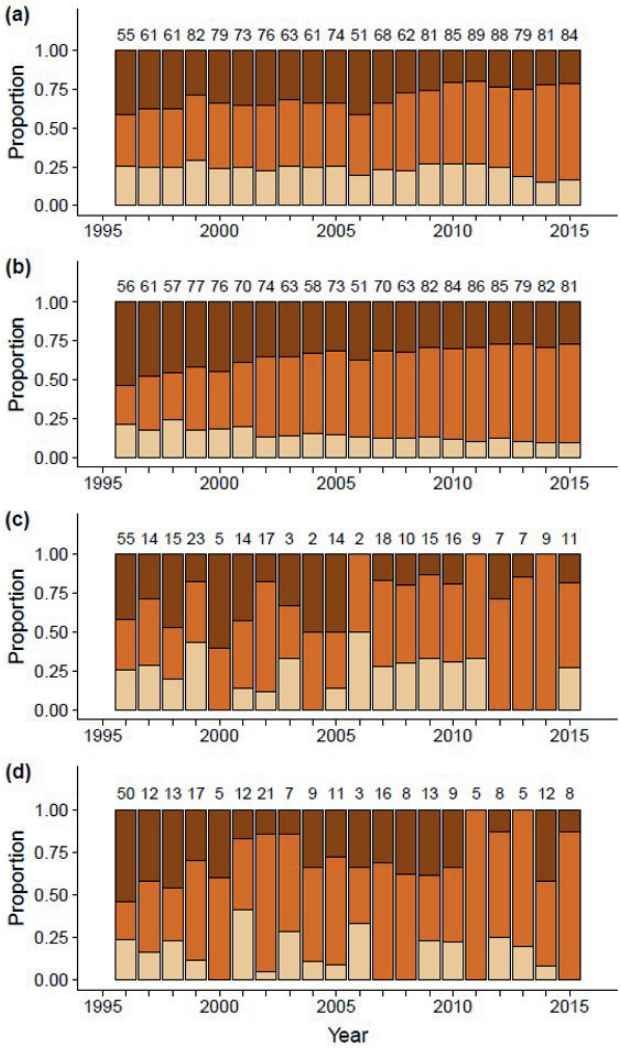

Figure 4.5: Yearly proportions of buzzards of the three plumage colour morphs between 1996 and 2015 (dark=brown, intermediate=orange, light=beige). We used the data from all individuals for which the morph had been identified in each year ($N_{females}$=1453 individual-years; N_{males}=1428 individual-years) and from all individuals in their first year of breeding only ($N_{females}$=266; N_{males}=244). (**a**) All females in the breeding population; (**b**) all males in the breeding population; (**c**) all females that bred for the first time; (**d**) all males that bred for the first time. Numbers on top indicate sample sizes.

Morph frequencies varied significantly among the years for both sexes, with intermediates becoming more frequent compared to the extreme morphs as the study progressed (figure 4.5, table S12, figure S4).

Discussion

A previous study on Common buzzards suggested that the colour polymorphism was maintained by heterosis, assuming a simple one-locus two-allele inheritance system with the intermediate morph being the heterozygote (Kruger et al 2001). This study showed that the intermediate colour morph had the highest fitness. In agreement with Krüger et al. (2001), we found that fitness (cumulative reproductive success) differed among morphs, with the intermediate morph having highest fitness. In contrast to this earlier study, however, we found a phenotypic change with an increasing proportion of intermediates over a 20-year period. This apparent evolutionary change did not just arise due to selection on individual phenotypes, but likely also from fitness benefits of assortative mating. As assortative pairs were more successful in raising chicks than disassortative pairs and assortatively paired intermediates produce higher percentage of intermediate offspring (74%) than by following a simple Mendelian inheritance system (50%) (Kappers et al. 2018), which could lead to a decline in frequencies of extreme phenotypes. This could lead to a positive feedback loop, for example if the extreme morphs take longer to find a suitable (assortative) mate, and ultimately to a decline in the extreme genotypes.

Because our study is a replication of the earlier studies on fitness consequences of colour polymorphisms in Common buzzards (Krüger et al. 2001; Boerner and Krüger 2009; Jonker et al. 2014), we first discuss differences and commonalities between the two studies, focussing on the different fitness components considered. These differences could arise through differences in ecological settings between the two populations, and hence the selection pressures on the buzzards, or through methodological differences.

In both studies, adult survival only differed minimally between the morphs, whereby the intermediate morph had slightly higher estimated annual survival (Fig. 1). However, overall annual survival was considerably lower in the German compared to the Dutch population (Jonker et al. 2014; Fig. 1). Both studies were based on large sample sizes and a long-term dataset and used similar methods for analysing annual survival. However, no (morph-specific) resighting rates have been reported for the German population. The method of individual identification of the breeding buzzards differed between the studies; the German study relied mostly on visual observations and photos, whereas we mostly used the unique banding patterns of moulted feathers. Whether this difference affects survival estimates remains unknown, but it seems unlikely that it could explain the much lower estimated survival rates in the German population. Individuals of the dark morph are probably most difficult to distinguish, but they constitute only 13% of the German population (Boerner & Krüger, 2009). Alternatively, selection pressures may be different in Germany, leading to lower local survival. This is not unlikely, given that the two populations differ in two relevant aspects. (1) The German population increased fourfold between 1989-

2015 (Mueller, Chakarov, Krüger & Hoffman, 2016), whereas the Dutch population was rather stable. (2) Eagle-owls (*Bubo bubo*) colonized the German area as predators of buzzards since 2003 (Mueller, Chakarov, Krüger & Hoffman, 2016), but are absent in the Dutch population. In addition, a constant effort to defend against poaching in the Dutch study area could have helped in maintaining a stable number of adults holding territories. Our survival estimates are comparable to those reported for adult Common buzzards from a UK population (88-91%, Kenward et al., 2000) and they are also similar to survival rates observed for other medium-sized hawks (reviewed by Newton, Mcgrady & Oli, 2016).

In the Dutch population, annual reproductive success was unrelated to morph. No comparative data have been published for the German population. However, in the Dutch population the mean number of fledglings seems about 50% lower than in the German population (Krüger, 2004). These data may not be directly comparable, because we included all pairs that held a territory, while Krüger (2004) only included breeding pairs.

In both populations, there is evidence for assortative mating for colour morph. Our study shows that assortative pairs were more likely to produce offspring, and that pair bonds lasted longer. In contrast, assortative mating in the German population was considered maladaptive, because under the hypothesis of simple Mendelian inheritance, light-dark pairs would produce 100% intermediate offspring with higher fitness (Krüger, Lindström & Amos, 2001). However, this simple inheritance patterns is not consistent with the data (Kappers et al., 2018). It remains unclear why assortative pairs in the Dutch population performed better, but it might be related to behavioural compatibility or to local habitat matching. Evidence for the former comes from a study on another polymorphic raptor, the black sparrowhawk, *Accipiter melanoleucus* (Tate, Sumasgutner, Koeslag & Amar, 2017). This study showed that neither of the two morphs had an advantage in terms of productivity or survival, but that the morph combination of adult pairs significantly influenced productivity. Mixed-morph pairs produced more offspring per year than same-morph pairs, possibly due to behavioural complementarity (Tate, Sumasgutner, Koeslag & Amar, 2017). Although in this case disassortative rather than assortative pairs had higher success, the study shows that pair-level fitness advantages may play an important role in promoting and maintaining a colour polymorphism in species with biparental care.

In both populations, there is clear evidence that long-term fitness measures differ between the morphs in favour of the intermediates. However, the effect sizes were much larger in the German population, where the intermediates produced at least twice as many fledglings during their lives compared to dark or light morphs (Boerner & Krüger, 2009). In our population intermediates had a 15% higher fitness. In the German population, the fitness differences between the morphs were due to both differences in mean life span and differences in reproductive success (Krüger, Lindström & Amos, 2001). However, we found no significant difference in reproductive success between the morphs after controlling for the number of breeding attempts (table S9).

Krüger, Lindström & Amos (2001) suggested that the higher fitness of individuals of the intermediate morph is due to (1) intermediates breeding in the highest quality territories, and (2) dark and light individuals having a lower breeding propensity. Hence, they suggested that the competitive advantage of individuals of the intermediate morph (Krüger,

2002), in combination with large variation in territory quality, resulted in the observed fitness advantage. Chakarov, Boerner & Krüger (2008) further suggested that the success of intermediate morphs could be related to parasite resistance. The study shows that buzzard nestlings with darker plumage were more susceptible to an ectoparasite (the carnid fly, *Carnus haemapterus*), while nestlings infected with a blood parasite (*Leucocytozoon toddi*) showed a higher infection intensity when they had lighter plumage. This suggests that the two parasite species might exert opposite selection pressures on plumage colour of the host, such that intermediate buzzards could have an advantage (Chakarov, Boerner & Krüger, 2008). However, the results depended on offspring sex and on food availability (vole density). Thus, the role of parasites in maintaining the colour polymorphism remains unclear. The lower fitness differences between the morphs in our population could be explained if territory quality is less variable in our study area. Given that competitive abilities may differ between morphs, it would be interesting to assess morph-dependent survival in the nest and post-fledging, and age at first breeding in relation to colour morph.

We set out to repeat a previous study and to explain the maintenance of the colour polymorphism in buzzards. We conclude that the mechanism suggested by Krüger, Lindström & Amos (2001) for the maintenance of this polymorphism (over-dominance) seems unlikely (see also Kappers et al., 2018). Instead, our results suggest that morph frequencies have changed directionally over the past years, with an increase in the proportion of intermediates. However, we failed to identify an ecological factor to explain this apparent evolutionary change. Intriguingly, in both populations intermediates seem to have a fitness benefit, suggesting a potential for evolutionary change. Nevertheless, these populations are still highly variable for this genetically determined trait. To solve this evolutionary paradox, we need a better understanding of the ecological causes behind the fitness differences. Several key pieces of information are still missing. First, we have no knowledge about morph-specific differences in survival until breeding, and in the likelihood to obtain a breeding territory. In the dimorphic juvenile mute swans *Cygnus cygnus*, the grey morph survived better, but started breeding later in life (Conover, Reese & Brown, 2000). Different buzzard morphs might have different early life-history strategies, countering the selection in favour of intermediate adult breeders. Second, we have little information about spatial variation in selection pressures on colour morphs (Gillespie & Turelli, 1989), and about phenotype-habitat matching (Edelaar, Siepielski & Clobert, 2008). There is ample evidence for clines or variation in colour morphs over larger (Antoniazza et al., 2010; Amar, Reynolds, Van Velden & Briggs, 2019) and smaller (Amar et al., 2014; Sordahl, 2014) spatial scales in raptors. However, there is relatively little evidence for a morph-by-habitat interaction on fitness (Dreiss et al., 2012). Our study clearly highlights that understanding the evolutionary dynamics in natural populations requires not only a long-term effort in monitoring a focal population, but also needs to include measures of fitness consequences that typically accrue outside the specific study site (dispersal and habitat choice, spatial variation in fitness parameters).

Acknowledgements

We thank the landowners for permission to work on their property and those who assisted in the field. We thank Rosemarie Kentie for advice on the mark-recapture analysis and Oliver Krüger and an anonymous reviewer for constructive comments.

Data accessibility

Data are available from the Open Science Framework at https://osf.io/s2z9r/.

Supplemental Material

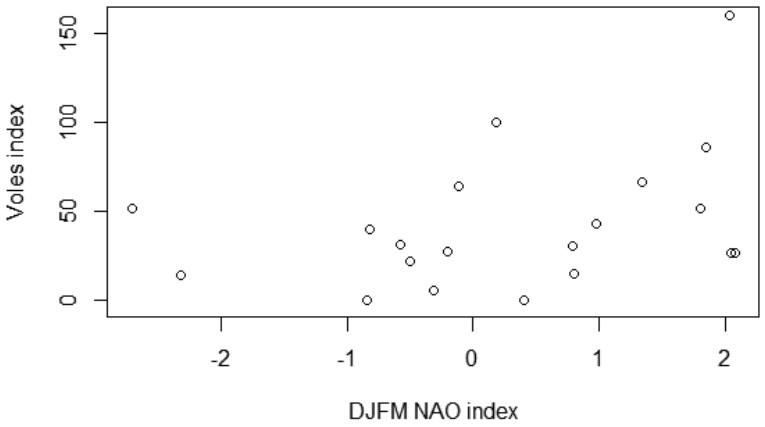

Figure S1: The relationship between the NAO index for December to March and the voles index during the study period 1996-2015 in the NE of The Netherlands (Pearson's r=0.36, N = 20, p=0.11). The North Atlantic Oscillation (NAO) index is based on the surface sea-level pressure difference between the Subtropical (Azores) High and the Subpolar Low. Negative values of the DJFM NAO index indicate colder winters.

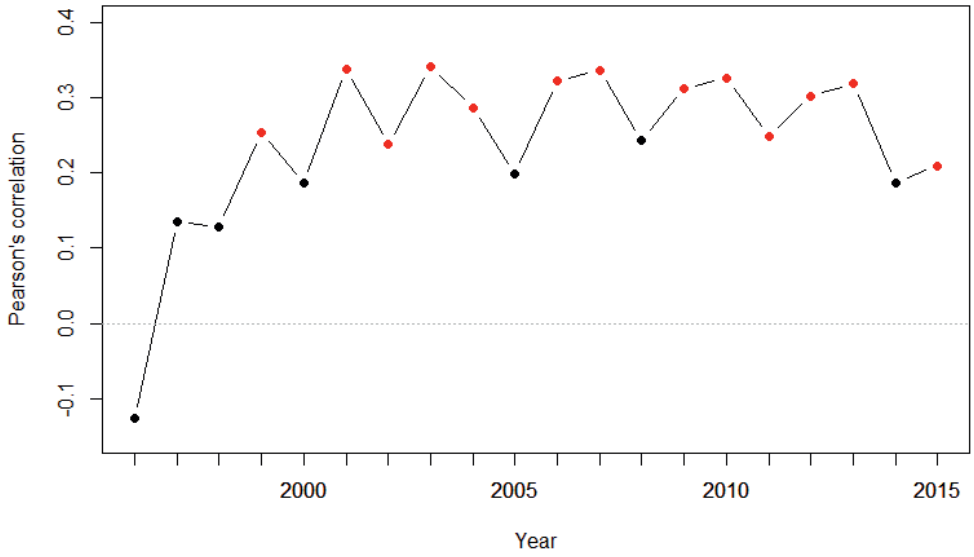

Figure S2: Pearson's correlation coefficients between the morph of the male and that of the female of a pair for all years of the study. The significance of the Pearson's correlation coefficients was tested by using a resampling procedure (100,000 simulations, see Materials and methods). Red dots indicate P<0.05.

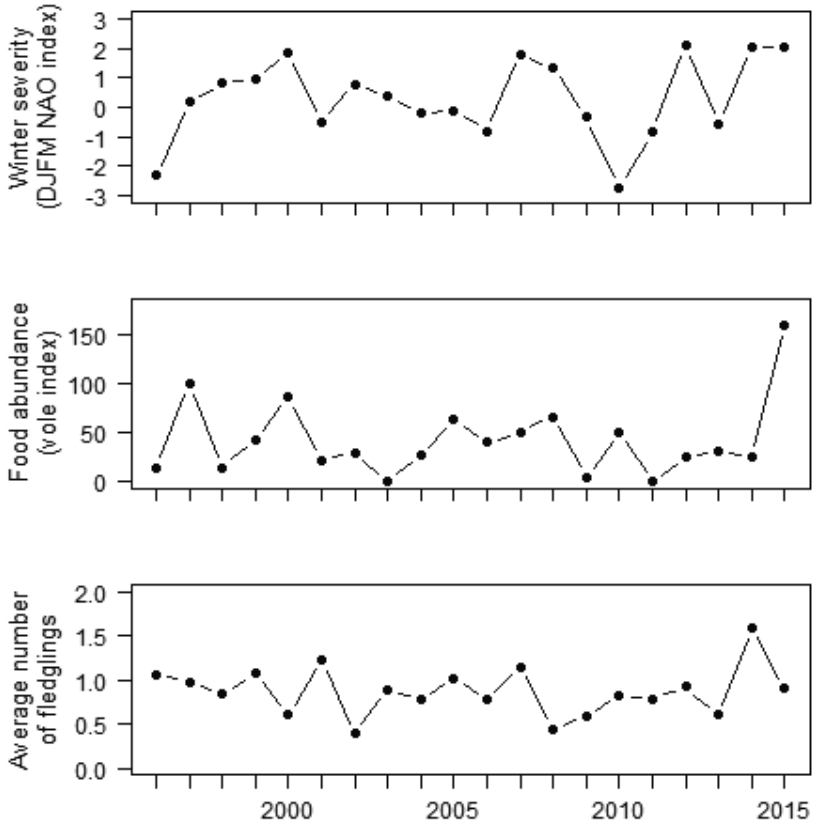

Figure S3: Yearly variation in environmental variables for the period 1996-2015 used in the capture-recapture analysis (see Methods and tables S2 and S3). Shown are the winter severity (upper panel, NAO index from December to March), food abundance (middle panel, vole index) and the average number of fledged chicks (bottom panel).

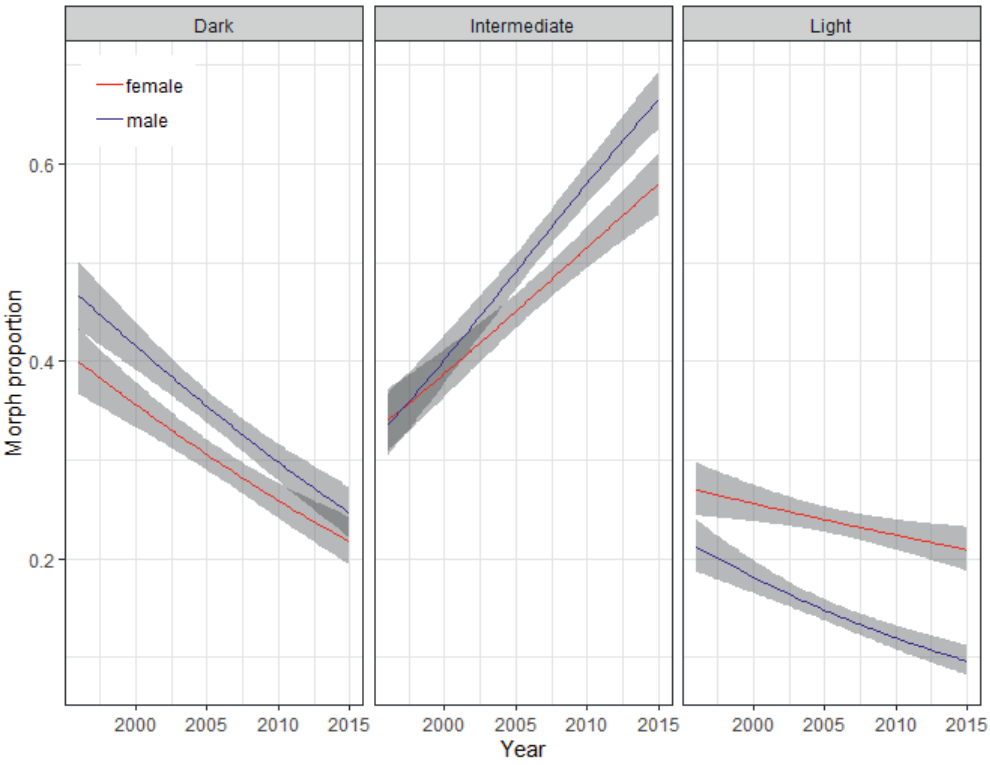

Figure S4: Proportion of all individuals of a given morph dependent on sex and year. Every morph was modelled using a GLM with binomial error distribution corrected for under-dispersion (i.e. using quasi-binomial family). See table S11. Shown are the model fit and the 95% confidence intervals.

Table S1: Results of a capture–recapture analysis for data on breeding male and female Common buzzards between 1996 and 2015. Buzzards are categorized by their colour morph (dark, intermediate and light). The analysis separates between survival probabilities (ϕ) that are kept constant (.), and re-sighting probabilities (p) that can be either constant (.), morph dependent (m) or year dependent (t). The three possible models are displayed in order of their fit to the data. Statistics given for each model include number of parameters, corrected Akaike information criterion (AICc), Delta AICc (that gives the difference in AICc between the current model and the best model), the proportional support of the model (i.e. the AICc weight) and the deviance. Models are corrected for overdispersion (Ĉ=1.77 for male and Ĉ=1.48 for female buzzards).

Model	*n*	AICc	DeltaAICc	Weight	Deviance
Males					
φ(.) p(t)	20	1128.624	0.0	0.4462	665.472
φ(.) p(.)	2	1129.014	0.39	0.380	702.487
φ(.) p(m)	4	1130.784	2.16	0.157	700.236
Females					
φ(.) p(t)	20	1942.118	0.0	0.999	1098.373
φ(.) p(.)	2	1977.501	35.38	0.000	1170.370
φ(.) p(m)	4	1981.450	39.33	0.000	1170.299

Table S2: List of candidate set of 24 models of a capture–recapture analysis for data on breeding male and female Common buzzards between 1996 and 2015. Buzzards are categorized by their colour morph (dark, intermediate and light). Survival probabilities (ϕ) can be either constant (.), or dependent on morph (m), year (t), voles abundance (v), fledgling success of the previous year (f), winter severity (w), and plausible interactions between these terms. Re-sighting probabilities (p) are year dependent (t).

Model number	Specification
1	***φ(.) p(t)***
2	***φ(m) p(t)***
3	***φ(t) p(t)***
4	***φ(v) p(t)***
5	***φ(f) p(t)***
6	***φ(w) p(t)***
7	***φ(t + m) p(t)***
8	***φ(w + m) p(t)***
9	***φ(v + f) p(t)***
10	***φ(v + m) p(t)***
11	***φ(f + m) p(t)***
12	***φ(v + w) p(t)***
13	***φ(f + w) p(t)***
14	***φ(m + v + w) p(t)***
15	***φ(m + w + f) p(t)***
16	***φ(m + v + f) p(t)***
17	***φ(f + v + w) p(t)***
18	***φ(m + v + f + w) p(t)***
19	***φ(v * m) p(t)***
20	***φ(f * m) p(t)***
21	***φ(v * w) p(t)***
22	***φ(w * m) p(t)***
23	***φ(v * f) p(t)***
24	***φ(t * m) p(t)***

Table S3: Output of the best models (ΔAICc <2) from a candidate set of 24 capture–recapture models for data on breeding male and female Common buzzards between 1996 and 2015. Buzzards are categorized by their colour morph (dark, intermediate and light). The analysis separates between survival probabilities (ϕ) that can be either constant (.), morph dependent (m), year dependent (t), dependent on winter severity (w), voles abundance (v), fledgling success (f) or plausible interactions among these variables, and recapture probabilities (p) that are year dependent (t). The best models are displayed in order of their fit to the data. Statistics given for each model include number of parameters, corrected Akaike information criterion (AICc), Delta AICc (that gives the difference in AICc between the current model and the best model), the proportional support of the model (i.e. the AICc weight) and the deviance. Models are corrected for overdispersion ($\hat{C}$ = 1.77 for male and $\hat{C}$ = 1.48 for female buzzards).

Model	*n*	AICc	DeltaAICc	Weight	Deviance
Males					
φ(.) p(t)	20	1128.624	0.0	0.21	665.47
φ(w) p(t)	21	1129.353	0.72	0.15	664.13
φ(v) p(t)	21	1130.495	1.87	0.08	665.27
φ(m) p(t)	22	1130.507	1.88	0.08	663.22
φ(f) p(t)	21	1130.687	2.06	0.07	665.47
Females					
φ(.) p(t)	20	1325.031	0.0	0.29	741.92
φ(f) p(t)	21	1326.333	1.30	0.14	741.16
φ(v) p(t)	21	1327.056	2.02	0.10	741.88
φ(w) p(t)	21	1327.093	2.06	0.10	741.92

Table S4: Pair bond duration (the number of years a pair bred in the population) in relation to the degree of pair assortment based on plumage morph (three levels: "0" for dark-light pairs as reference category, n=36; "1" for intermediate-dark or intermediate-light pairs, n=192; and "2" for pairs where both partners had the same morph, n=172).

Predictors		**Estimate**	***SE***	***P***
Intercept		0.45	0.14	
Assortative mating score: 1		0.50	0.13	**<0.001**
Assortative mating score: 2		0.55	0.13	**<0.001**
Random Effects				
σ^2	0.49			
$\tau_{00\ female\ ID}$	0.03			
$\tau_{00\ male\ ID}$	0.00			
$\tau_{00\ startYear}$	0.09			
$ICC_{female\ ID}$	0.06			
$ICC_{male\ ID}$	0.00			
ICC_{year}	0.14			
Observations	400			
Marginal R^2/Conditional R^2	0.036/0.228			

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient

Table S5: Pair bond duration (the number of years a pair bred in the population) in relation to pair morph in positive assortative mated pairs (two levels: "Extremes" for dark-dark and light-light pairs as reference category, n=64 of which $n_{D\text{-}D}$=41 and $n_{L\text{-}L}$=23; "Intermediates" for intermediate-intermediate pairs, n=108).

Predictors		**Estimate**	***SE***	***P***
Intercept		1.08	0.11	
Intermediates		-0.09	0.12	0.48
Random Effects				
σ^2	0.32			
$\tau_{00\ female\ ID}$	0.03			
$\tau_{00\ male\ ID}$	0.13			
$\tau_{00\ startYear}$	0.08			
$ICC_{female\ ID}$	0.05			
$ICC_{male\ ID}$	0.23			
ICC_{year}	0.14			
Observations	172			
Marginal R^2/Conditional R^2	0.003/0.419			

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient

Table S6: Results of a GLMM model testing for variation in individual annual reproductive output dependent on morph of both attendant adults ("intermediate" as reference category), the degree of assortment by morph of the pair (three levels: "0" for dark-light pairs as reference category, n=93; "1" for intermediate-dark or intermediate-light pairs, n=674; and "2" for pairs where both partners had the same morph, n=592), and disturbance (3 levels, "no evidence of disturbance" as reference category). We defined yearly reproductive success as the number of fledglings produced in that year for all nests where the morph of both parents was known.

Predictors	**Estimate**	***SE***	***P***
Intercept	-0.36	0.19	
Male morph: Dark	0.06	0.09	0.47
Male morph: Light	-0.01	0.12	0.89
Female morph: Dark	0.08	0.09	0.41
Female morph: Light	0.07	0.11	0.51
Assortative mating score: 1	0.11	0.16	0.48
Assortative mating score: 2	0.30	0.17	0.07
Disturbance: Egyptian goose	-0.95	0.38	**0.013**
Disturbance: Human	-1.05	0.11	**<0.001**
Random Effects			
σ^2	0.86		
$\tau_{00\ female\ ID}$	0.08		
$\tau_{00\ male\ ID}$	0.08		
$\tau_{00\ year}$	0.10		
$ICC_{female\ ID}$	0.07		
$ICC_{male\ ID}$	0.07		
ICC_{year}	0.09		
Observations	1359		
Marginal R^2/Conditional R^2	0.132/0.334		

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient

Table S7: Results of GLMM models testing for variation in individual annual reproductive output as nest success (left) and fledgling productivity of successful nests (right) dependent on morph of both attendant adults ("intermediate" as reference category), the degree of assortment by morph of the pair, and disturbance (3 levels, "no evidence of disturbance" as reference category).

	Nest success			**Fledgling productivity (of successful nests)**		
Predictors	**Estimate**	***SE***	***P***	**Estimate**	***SE***	***P***
Intercept	-0.19	0.42		0.49	0.14	
Male morph: Dark	0.19	0.21	0.36	0.05	0.06	0.43
Male morph: Light	-0.09	0.28	0.74	0.01	0.09	0.91
Female morph: Dark	0.11	0.21	0.60	0.03	0.06	0.60
Female morph: Light	0.02	0.24	0.92	0.08	0.07	0.30
Assortative mating score:1	0.26	0.36	0.46	0.03	0.13	0.79
Assortative mating score:2	0.77	0.36	**0.036**	0.05	0.13	0.67
Disturbance: Egyptian goose	-1.64	0.63	**0.009**	-0.21	0.38	0.57
Disturbance: Human	-2.16	0.21	**<0.001**	-0.08	0.11	0.45
Random Effects						
σ^2	3.29			0.45		
$\tau_{00\ female\ ID}$	0.41			0.00		
$\tau_{00\ male\ ID}$	0.50			0.00		
$\tau_{00\ year}$	0.37			0.01		
$ICC_{female\ ID}$	0.09			0.00		
$ICC_{male\ ID}$	0.11			0.00		
ICC_{year}	0.08			0.02		
Observations	1359			732		
Marginal R^2/Conditional R^2	0.138/0.379			0.004/0.026		

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient

Table S8: Cumulative reproductive success (CRS) for females and for males, as the total number of fledglings produced during their presence in the population, dependent on morph of each parent ("intermediate" as reference category). CRS was modelled using a GLMM with a Poisson distribution, a log-link and a Laplace approximation.

	Female CRS			**Male CRS**		
Predictors	**Estimate**	***SE***	***P***	**Estimate**	***SE***	***P***
Intercept	1.26	0.18		1.48	0.11	
Dark morph	-0.24	0.06	**<0.001**	-0.13	0.06	**0.040**
Light morph	-0.11	0.07	0.11	-0.16	0.08	**0.044**
Random effect						
σ^2		0.27		0.22		
τ_{00}		$0.61_{StartYear}$		$0.22_{StartYear}$		
ICC		$0.69_{StartYear}$		$0.51_{StartYear}$		
Observations		266		244		
Marginal R^2/Conditional R^2		0.012/0.698		0.012/0.514		

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient

Table S9: Cumulative reproductive success (CRS) for females and for males, as the total number of fledglings produced during their presence in the population, dependent on morph of each parent ("intermediate" as reference category). CRS was modelled using a GLMM with a Poisson distribution, a log-link and a Laplace approximation, on the subset of individuals that were not missing for more than 2 years from the population.

	Female CRS			**Male CRS**		
Predictors	**Estimate**	***SE***	***P***	**Estimate**	***SE***	***P***
Intercept	1.24	0.18		1.49	0.12	
Dark morph	-0.28	0.06	**<0.001**	-0.15	0.06	**0.018**
Light morph	-0.13	0.07	0.07	-0.15	0.08	0.97
Random effect						
σ^2		0.27		0.22		
τ_{00}		$0.63_{StartYear}$		$0.25_{StartYear}$		
ICC		$0.70_{StartYear}$		$0.53_{StartYear}$		
Observations		251		225		
Marginal R^2/Conditional R^2		0.015/0.703		0.013/0.538		

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient

Table S10: Cumulative reproductive success (CRS) for females and for males, as the total number of fledglings produced during their presence in the population, dependent on morph of each parent ("intermediate" as reference category) and on the number of breeding attempts as covariate. CRS was modelled using a GLMM with a Poisson distribution, a log-link and a Laplace approximation.

	Female CRS			**Male CRS**		
Predictors	**Estimate**	***SE***	***P***	**Estimate**	***SE***	***P***
Intercept	0.22	0.09		0.60	0.05	
Dark morph	-0.01	0.06	0.89	0.05	0.05	0.36
Light morph	-0.02	0.07	0.68	0.05	0.08	0.97
N of breeding attempts	0.15	0.005	**<0.001**	0.12	0.003	**<0.001**
Random effect						
σ^2	0.27			0.22		
τ_{00}	$0.10_{StartYear}$			$0.00_{StartYear}$		
ICC	$0.26_{StartYear}$			$0.00_{StartYear}$		
Observations	266			244		
Marginal R^2/Conditional R^2	0.631/0.728			0.665/0.665		

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient

Table S11: Lifetime reproductive success (LRS) for females and for males, as the total number of fledglings produced in their lifetime, dependent on morph of each parent ("intermediate" as reference category). LRS was modelled using a GLMM with a Poisson distribution, a log-link and a Laplace approximation, on the subset of individuals that were assumed dead after not having been observed during three consecutive breeding seasons

	Female LRS			**Male LRS**		
Predictors	**Estimate**	***SE***	***P***	**Estimate**	***SE***	***P***
Intercept	0.40	0.21		0.73	0.22	
Dark morph	-0.11	0.10	0.30	-0.29	0.10	**0.003**
Light morph	0.03	0.11	0.77	-0.06	0.11	0.60
Random effect						
σ^2	0.52			0.42		
τ_{00}	$0.55_{StartYear}$			$0.56_{StartYear}$		
ICC	$0.52_{StartYear}$			$0.57_{StartYear}$		
Observations	161			143		
Marginal R^2/Conditional R^2	0.003/0.518			0.018/0.578		

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient

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Table S12: Proportion of a given morph from the total dependent on sex and year. Every morph was modelled using a GLM with binomial error distribution corrected for under-dispersion (i.e. using quasi-binomial family). Dispersion parameter were 0.387, 0.407 and 0.316 for the models on proportion of dark, intermediate and light morph respectively. Shown are the non-parametric bootstrap confidence intervals (CI).

Predictors	**Estimate**	***SE***	***P***	**2.5% CI**	**97.5% CI**
Proportion of dark morph individuals					
Intercept	91.31	12.48	<0.001	63.23	113.7
Year	-0.045	0.006	<0.001	-0.057	-0.032
Sex: Male	11.74	17.45	0.51	-21.17	47.15
Year*Sex: Male	-0.005	0.008	0.51	-0.023	0.010
Proportion of intermediate morph individuals					
Intercept	-1.04×10^{2}	1.19×10^{1}	<0.001	-129.9	-81.68
Year	5.19×10^{-2}	5.91×10^{-3}	<0.001	0.040	0.064
Sex: Male	-4.06×10^{1}	1.70×10^{1}	0.02	-79.81	-2.108
Year*Sex: Male	2.03×10^{-2}	8.47×10^{-3}	0.02	0.0011	0.039
Proportion of light morph individuals					
Intercept	34.020	12.01	0.007	2.375	65.33
Year	-0.017	0.006	0.006	-0.0332	-0.0017
Sex: Male	62.177	19.05	0.002	21.67	100.6
Year*Sex: Male	-0.031	0.0095	0.002	-0.050	-0.011

Appendix 1. Identification of individuals of the polymorphic Common buzzard using phenotypic characteristics of shed feathers.

Female Common buzzards start moulting their flight feathers in the late nestling rearing phase, whereas males typically moult between July and October, after the bulk of provisioning of the offspring has been completed (Dare 2015). Between 1995 and 2012, we collected 205 shed feathers from 10 occupied breeding territories. Feathers were found below occupied nests and at nearby perches between June and October. All shed feathers (primaries, secondaries, tertials, tail feathers and upperwing coverts) came from mature birds (5 females and 5 males) and were stored at room temperature in paper envelopes according to site and date of collection. We compared feathers collected in the same territory in different years (4 years for 8 territories, 5 years for 2 territories; on average 4.9 feathers per year per territory, range: 1-5). Buzzards in the study population are resident, persistently use the same nesting areas and only breed once a year, hence are unlikely to be represented at multiple sites in the same year (see also Dare, 2015).

To identify adult individuals, we compared feathers from year to year using three phenotypic characteristics: feather length, colour and pattern of pigmentation (figure A1).

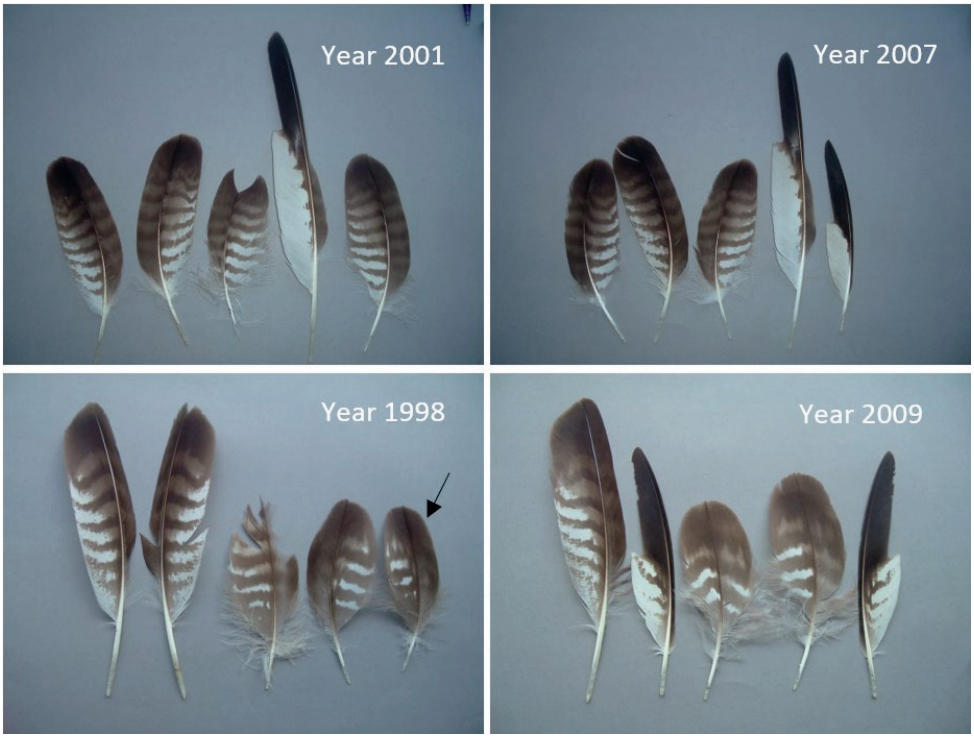


Figure A1: Two examples of individual identification based on characteristics of moulted feathers. Dorsal side of moulted feathers collected at the same nest site in different years for a light morph female (top row) and a dark morph female (bottom row). The arrow points at a misidentified feather.

We extracted DNA from a 5 mm clipping of the basal calamus tip and a 1 cm slice of the calamus wall just below the superior umbilicus (blot clot region, see Horwáth et al. 2005). The material was chopped down to small pieces in a digestion solution containing 300 µl TNE (10mM Tris-HCl, 100mM NaCl, 10mM EDTA; pH8), 25 µl Proteinase K (25 mg/ml), 15 µl 20% SDS and 20 µl freshly prepared 1 M DTT. The material was incubated at 56 °C until completely lysed (overnight up to 24 h). If necessary, more DTT and/or Proteinase K was added. From this lysate the DNA was purified with a standard phenol-chloroform extraction method (e.g. Sambrook 1989) and subsequent ethanol precipitation. Finally, the DNA was dissolved in 40 ul TE buffer.

We genotyped all samples at twelve microsatellite loci (and one sex chromosome-linked marker, table A1). Microsatellite amplifications were performed in multiplexed PCRs using the Qiagen Type-it Microsatellite PCR Kit and primer mixes containing six or seven primer pairs (mix 1 and 2; table A1). The forward primer of each pair was fluorescently labelled with 6-FAM, VIC, PET or NED (Dye Set G5, Thermo Fisher Scientific). Differences in amplification efficiency and dye strength of the primers were accommodated by adapting the primer concentrations in these mixes (details given in table A1). Each 20 µl multiplex PCR contained 2 ul of above DNA extract, 10 µl of the 2x Type-it Microsatellite PCR Master Mix and 2 µl of one of a primer mix. Cycling conditions were: 5 min initial denaturation at 95 ˚C, 35 cycles of 30 s denaturation at 94 ˚C, 90 s annealing at 53 °C (primer mix 1) or 51 °C (primer mix 2), and 1 min extension at 72 ˚C, followed by a 30 min completing final extension at 60 ˚C. After amplification, 3 µl of the PCR products were added to 13ul formamide containing the GeneScan 500 LIZ Size Standard, heat denatured and resolved in POP4 polymer on an ABI 3130 Genetic Analyzer. Raw data were analysed and alleles assigned using the GENEMAPPER 4.0 software.

We successfully genotyped 97% (199/205) of all feathers at 6-12 loci (mean: 11.8). Between 2 and 9 alleles were scored per locus, with an average of 4.8 alleles per marker. For 8 territories, all collected feathers (n=20 per territory) that were phenotypically identified as being from the same individual, showed complete genotype matching. For the remaining two territories with a total of 20 and 21 feathers collected, respectively, the genotype of two feathers (one in each territory) did not match with that of the other feathers found in the same location (n=10 and 11 mismatches at 12 genotyped loci for the two feathers, respectively). Thus, we concluded that these two feathers came from a different individual. Overall, the genetic markers suggested that the 199 samples came from 12 unique individuals. The false positive error rate of feather assignment (feathers wrongly assigned to the same individual based on phenotype) was 2/199 = 1%.

Table A1: Details of microsatellite and sex chromosomal markers used to genotype feathers from 10 nesting territories of the Common buzzard *Buteo buteo*. "Label" refers to the fluorescent label used for the forward primer; *C* refers to primer concentration in the multiplex primer mix 1 or 2. T_a = the annealing temperature; *n* = the number of alleles.

Locus	Reference	Label	Primer sequences (5' - 3')	*C* (µM)	Multiplex Mix	T_a (°C)	Size range (bp)	*n*
Bbu03	Johnson et al. (2005)	PET	GATCAAAGTACTTGA CAGTGTCCCAGGTAC ATGCGTACATACTTC	0.39 µM	1	53	209 - 217	5
Bbu06	Johnson et al. (2005)	PET	ACCAGTTCCATTCTG CTTGCTTACTTGAAA CTGTAAACCTTCGTT G	0.24 µM	2	51	112 - 118	3
Bbu11	Johnson et al. (2005)	VIC	ACTTCACTTATGAAA ACAGACCAAATCACC AGGTTGCAGCTGAGT G	0.15 µM	1	53	127 - 131	3
Bbu17	Johnson et al. (2005)	PET	GAGGTCACTGGCTC GAGATGGCATTTTGC TTTGGATTTAAGC	0.17 µM	2	51	171 - 177	4
Bbu14	Johnson et al. (2005)	6FAM	CAAATGTTCTCAACA GCTTAAGTCCTCATT ACTACTGTTAGAAAT AGGCTTG	0.18 µM	1	53	131 - 139	3
Bbu30	Johnson et al. (2005)	PET	GACCAGAAGCCTTGA CTTGCTTTGCTTCCT GAATAGGATGG	0.2 µM	1	53	153 - 157	3
Bbu33	Johnson et al. (2005)	VIC	TGCCGCCATCTTACT GAAGATCACAAGATA GCCAGCTATGG	0.28 µM	1	53	160 - 168	5
Bbu34	Johnson et al. (2005)	NED	AGACCAGCAAACCCA AACAGTTGATATATC TTGCTCCATGCTG	0.17 µM	2	51	143 - 157	8
Bbu42	Johnson et al. (2005)	6FAM	GGGATAAGAATGCCA GAACTTGTGGGTGG CTAAATCTTGAGG	0.15 µM	2	51	140 - 182	9
Bbu46	Johnson et al. (2005)	6FAM	TGAACCCTGGAGAAA GATGCCAATTTGGGG AGACGTGATG	0.14 µM	1	53	155 - 189	6
Bbu51	Johnson et al. (2005)	VIC	GACCTGGTGCTCTGC ATTCTGAAACAGATT TGATTCTGGATG	0.32 µM	2	51	148 - 170	7
Bbu59	Johnson et al. (2005)	NED	CCTGCCACAGGGTAT TACTATGACAGGCTC GCTAAAGGAACAAG	0.14 µM	1	53	130 - 132	2
3007/3112	Ellegren & Fridolfsson (1997)	PET	TACATACAGGCTCTA CTCCTCCCCTTCAGG TTCTTTAAAA	0.7 µM	2	51	380 – 386	2

5

Colour polymorphism predicts exploratory behaviour but not habitat choice during natal dispersal in a raptor species

Elena Frederika Kappers
Mihai Valcu
Christiaan de Vries
Anneke Alberda
Christiaan Both
Bart Kempenaers

Manuscript

Abstract

Colour polymorphism in animals can be maintained by selection pressures that may vary in time and space depending on specific ecological settings. Although the effects of colour variation on several life-history traits are well described, effects on natal dispersal behaviour are understudied. Moreover, effects of colour polymorphism on space use strategies throughout the early stages of life are lacking. Here, we studied the effects of plumage coloration on natal dispersal behaviour in the polymorphic Common buzzard (*Buteo buteo*). Furthermore, we studied the effect of coloration on habitat choice in the first months of wandering. Using GPS transmitter data collected from 64 juveniles in a Dutch population, we tested whether plumage coloration influenced emigration timing, number of areas visited, tenure in areas, cumulative distance among areas, distance of settlement from nest in first winter and proportion of forested habitat chosen. We found that coloration was only associated with the number of areas visited, but not with other traits. Darker individuals visited a higher number of areas during the first months of dispersal compared to lighter individuals. Our results highlight the importance of understanding ecological and social selection pressures acting on colour polymorphic species in such an important but yet poorly understood life-history stage as the natal dispersal.

Introduction

Genetically determined colour morphs are common across the animal kingdom, but the selection pressures responsible for the maintenance of this genetic variation are often poorly understood. These selection pressures are likely to be frequency or habitat dependent, and may vary across time and space depending on the specific ecological settings. Additionally, colour variation could be linked to other physiological and behavioural trait variation, with selection acting on a suite of linked traits. Indeed, behavioural traits are often associated with variation in melanin-based coloration because genes encoding for colour pleiotropically influence physiology (Ducrest et al. 2008; Van den Brink et al. 2012; Van Den Brink et al. 2012). Again, selection should vary on these trait combinations in space or time, depending on variable ecological conditions (like frequency-dependence or density dependence) in order to maintain this variation.

Numerous studies have shown differential life-history traits in melanin-based polymorphic species (see Roulin 2004), which leads to the hypothesis that morphs might be adapted to different environments that may vary in food abundance, social interactions or climate. It has also been suggested that colour morphs could influence movement ecology and in particular dispersal behaviour because of the association of boldness/exploration with dispersal on the one hand and with coloration on the other (van den Brink et al. 2012; Saino et al. 2014; Camacho 2018).

As selection on colour morphs may depend on habitat features and hence may vary in a spatial context, it is important to understand how individuals varying in morph behave spatially. Polymorphic species can be locally adapted in heterogeneous environments and show differential spatial distribution on a small scale (Preston 1980; Dreiss et al. 2012), leading to phenotype-habitat matching (Edelaar et al. 2008). Morphs can prefer habitats that provide protection from predators by visually matching backgrounds (Ahnesjö and Forsman 2006), or that increase hunting success by favourable light conditions (Tate et al. 2016; Tate and Amar 2017). Habitats that provide visually matching backgrounds might be preferred by raptors to conceal them from prey, although support is rather controversial (Nebel et al. 2019).

Whereas most research on space use of colour polymorphic birds has been done on adults, there is little knowledge on space use strategies at the time when selection is strongest: during the period between independence and recruitment to the breeding population. At this stage, individuals often sample different areas, and need to decide where they can settle depending on factors like their own morph, the habitat type, but also the competition from other young, and especially adult territorial individuals. The aim of this study is to understand whether dispersal traits in the months after independence are related to colour morph in Common buzzards (Buteo buteo), a raptor with continuous and highly heritable variation in plumage colour.

Natal dispersal is a key life-history trait because it affects population dynamics and population genetic structure (Whitlock 2001). Interestingly, spatial behaviour such as dispersal is often part of a behavioural syndrome (Dingemanse et al. 2003; Duckworth and Kruuk 2009; Hawkes 2009; Kurvers et al. 2009; Cote et al. 2010; Vegvari et al. 2011; Patrick and Weimerskirch 2014), which could also be genetically linked to melanin-based colour

variation (Ducrest et al. 2008; Van den Brink et al. 2012; Van Den Brink et al. 2012). Generally, darker individuals are often bolder, more explorative and more resistant to stress than lighter ones (Mafli et al. 2011; Mateos-Gonzalez and Senar 2012; Schweitzer et al. 2015). As a consequence, species displaying different colour morphs may differ in dominance and aggressive interactions and colouration could thus in turn affect habitat selection and territorial behaviour (Kallioinen et al. 1995; Lank et al. 1995; Sinervo and Zamudio 2001; Tuttle 2003; Pryke and Griffith 2006; Brazill-Boast et al. 2013).

The association between coloration and dispersal has been poorly investigated so far. Studies found that Barn swallows (*Hirundo rustica*) and Pied flycatchers (*Ficedula hypoleuca*) with darker colour pigmentation were more likely to disperse (Saino et al. 2014; Camacho 2018). However, other studies found no difference in distance of first breeding site from the natal nest between morphs of Barn owl (*Tyto alba*), Eleonora's falcon (*Falco eleonorae*) and Black sparrowhawk (*Accipiter melanoleucus*) recruits (Emaresi et al. 2014; Sumasgutner et al. 2016; Gangoso and Figuerola 2019).

In this study, we tested the hypothesis that variation in melanic coloration predicts natal dispersal behaviour in the Common buzzard. The general aim of our study is to improve our knowledge on the selection pressures acting on colour morphs. Common buzzards are highly variable in plumage coloration (Ulfstrand 1970; Kappers et al. 2017) and earlier studies showed that this colour polymorphism influences fitness of adult individuals (Krüger et al. 2001, Kappers et al. 2020). Recently, we showed a phenotypic change with an increasing proportion of intermediate morphs over time (Kappers et al. 2020). Intermediate morphs had higher cumulative reproductive success and assortative pairs were more likely to produce offspring than disassortative pairs (Kappers et al. 2020). We concluded that apparent evolutionary change not just arose from selection on individual phenotypes, but also from fitness benefits of mating. However, our fitness estimates did not include dispersal and recruitment, a period in which selection is likely to be strongest.

We used satellite-telemetry to track 72 juvenile Common buzzards during their first months of dispersal to test whether their movement patterns and spatial distribution differed among morphs. Studies of natal dispersal in raptors have focused on different developmental stages: a post-fledging dependence period ending in emigration from the natal environment, a transitional phase with provisional settlement in temporary areas (also called transient or wandering stage), and settlement at the first breeding site. We focus on the transient stage. Telemetry methods now allow to measure where individuals establish more or less stable home ranges during this stage. Under the hypothesis that darker buzzards are bolder and more explorative than lighter buzzards (Ducrest et al. 2008), we predicted that darker morphs 1) emigrate earlier, 2) explore more areas, 3) travel over longer distances and 4) settle further from their natal area in their first winter. Moreover, as Common buzzard morphs may disperse to find the best habitat conditions to which they are adapted, and we reasoned that dark individuals would benefit from crypsis mostly in concealed habitats, we predict that darker morphs would visit areas with a higher proportion of forested habitat. Studies of dispersal in raptors found that females disperse further than males (Newton and Marquiss 1983; Korpimäki 1993), as males tend to be philopatric to increase chances of territory acquirement, whereas females tend to disperse to get mates and resources necessary to breed successfully (Johnson and Gaines 1990). In

our buzzard population, we tested the hypothesis that females would emigrate earlier and settle further than males. Our study tests these predictions and describes in detail the dispersal process in the first months after emigration.

Materials and methods

Study species and site

The Common buzzard is a long-lived raptor that may disperse over several years before the first reproduction attempt occurs (Dare 2015). During juvenile dispersal, individuals often settle in different home ranges over time and acquire skills such as becoming more efficient in hunting (Walls and Kenward 1997; Dare 2015).

We studied Common buzzards from a long-term study population in Friesland, The Netherlands. The study site encompasses an area of 5724 ha with 1400 ha of forested patches, centred at 53°04'09.2"N, 6°13'46.6"E, and contained on average 76 ± 12 SD breeding pairs/year over a 20-year period (1996–2015). Monitoring Common buzzards throughout this study area consisted of visits in February and March to confirm any pre-breeding activity (e.g., presence of the adult pair, display flights, nest building, or copulation), during the incubation and early nestling period in April-May to confirm breeding attempts, prior to fledging to ring the nestlings, and after fledging to determine breeding success (i.e., number of fledged chicks).

Field procedures

During 2015 and 2016, we monitored 36 active nests before and during the nestling phase and measured weight and wing length of the nestlings up to four times (mean: 2.6 times). We determined nestling sex with genetic analyses using primers 3007 and 3112 (Ellegren and Fridolfsson 1997) on growing feathers collected during the various nest checks.

We estimated nestling age based on the following formula obtained from growth curves (Bijlsma 1997, 2000): y = ax4 + bx3 + cx2 + dx + e, where x is the wing length of an individual (the other values depend on sex and are a= -8.42*10-9, -9.69*10-9; b= 7.95*10-6, 8.5*10-6; c= -2.39*10-3, -2.41*10-3; d= 0.386, 0.378; e= -5.14, -4.72 respectively for females and males). We also calculated a scaled body condition index, following Peig et al. (2009), as follows: $\hat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{bSMA}$, where M_i and L_i are the body mass and the linear body measurement of individual i respectively; bSMA is the scaling exponent estimated by the SMA regression of M on L; L_0 is an arbitrary value of L (e.g. the arithmetic mean value for the study population); and $\hat{M}_i$ is the predicted body mass for individual i when the linear body measure is standardized to L0.

We scored plumage coloration according to a seven-morph scheme (Kappers et al. 2017). We also re-categorized plumage coloration using a three-morph scheme following Kappers et al. (2017) to be consistent with previous studies (e.g. Krüger et al. 2001). We used both morph schemes in our analyses (see supplementary material for results with the three-morph scheme).

Tagging

We fitted satellite transmitters to 34 juveniles in 2015 and to another 38 individuals in 2016. Individuals were estimated to have hatched between 29 May and 22 June 2015 (mean: 10 June), and between 2 and 23 June 2016 (mean: 14 June). In total, we tagged 45 males and 27 females. The tagged juveniles came from 36 different nests and 26 different pairs (i.e. nestlings from 10 pairs were tagged in two years). All tagged individuals were between age 30 and 39 days (mean ±SD: 34.8 ±1.9), i.e. when the body is almost fully-grown, the morph can be scored, and there is no risk that they would jump out of the nest (Bijlsma et al. 1994; Bijlsma 1997). We never observed nest desertion or brood failure in relation to capture events.

Of the 72 juveniles, 12 were dark, 12 were dark-intermediate, 18 were intermediate, 8 were light-intermediate, 15 were light and 7 were very light (we had no “very dark” individuals). This translates to 12 dark, 38 intermediate and 22 light individuals according to the three-morph scheme.

We used three solar-powered transmitter models: 25 g GPS-GSM tags from Microwave Telemetry Inc. (n=30 + 9 reused), 23 g GPS-GSM tags from Ecotone Telemetry Inc. (n=4) and 19 g GPS-GSM tags from Ecotone Telemetry Inc. (n=29).

We fitted transmitters with a backpack harness made out of a body loop directly connected to a neck loop using 25'' tubular Teflon Ribbon (Bally Ribbon Mills, Bally, Pennsylvania). At the time of tagging, individuals weighed between 667 and 984 g (mean ±SD: 788 g ±74). Thus, transmitter weights were less than 3–5% of the body weight, as recommended (Kenward 2000).

We programmed each transmitter to record data between sunrise and sunset, i.e. with a duty cycle such that GPS locations were only collected during periods when birds move. Locations were obtained at intervals ranging from 60 per hour to 1 per day, depending on the battery level and transmitter model (mean locations per day ±SE: 5.6 ±0.62 for Ecotone TI and 10.2 ±1.06 for Microwave TI). During winter months with low sunlight (October to December), batteries did not charge enough to send at least one position per day, but we could follow the individuals again when day length started to increase (January-February) (mean locations per active day ±SD: 16 ±28, range 1–383). The tags of 26% of the juveniles were still sending positions one year after tagging.

Emigration

Date of emigration was defined as the date when an individual had moved >0.54 km from its nest without returning for 10 consecutive days. We chose this distance threshold based on the average distance between nests of first-order neighbours in the 2016 breeding season (we assumed a similar breeding density in 2015; see Kappers et al. 2020). We determined the first-order neighbours of each breeding pair, by constructing Voronoi polygons and calculating the average distance-from-natal-nest. Given the coordinates of the nests, we computed a maximum likelihood estimate of the total study area that is the convex hull of the points (following Ripley and Rasson 1977) with the function *ripras* of the package *spatstat* (Baddeley and Turner 2004) in R (R Core Team 2016). We chose ten days as the minimum time an individual had to be away from the territory (even if it returned later),

because a study on Golden eagles *Aquila chrysaetos* showed that this is the maximum period a juvenile can survive without food from its parents (Weston et al. 2013).

For eight juvenile buzzards the transmitter stopped sending locations before they emigrated (three individuals in June, four in July and one in September). We assumed the juveniles had died, but only for one we could retrieve the transmitter in the vicinity of the nest with signs of predation.

Thus, the final dataset consisted of 64 buzzards (24 females, 40 males), of which 12 were dark, 10 dark-intermediate, 16 intermediate, 8 light-intermediate, 14 light and 4 very light (three-morph scheme: 12 dark, 34 intermediate, 18 light). The birds that did not disperse were 2 dark-intermediate, 2 intermediate, 1 light and 3 very-light (three-morph scheme: 4 intermediate and 4 light). Using the data from these birds, we ran a linear mixed model including emigration date (Julian) as response variable and sex (factor), morph (continuous, scaled by subtracting the mean and dividing by the standard deviation) and their second-order interaction as fixed effects. We also included year (factor), hatching date (continuous, scaled), body index (continuous, scaled) and brood size at fledging (continuous) as covariates. Because the data included siblings, we included the identity of the nest (n=36) and the parents (n=26) as random effects. As the interaction term was not significant (see table 5.1 and S1) we did not retain it in the final model. Before performing the analyses, we confirmed that there was no collinearity among our predictors.

Dispersal behaviour

We described dispersal behaviour of all 64 individuals between the date of emigration until 200 days after tagging (until circa beginning of January). We chose this time window because it was the period for which we had the biggest sample size, both in terms of individuals and locations. During the selected period, the mean period an individual was followed before the tag stopped sending data was 90 days ±SD 34 (range 15–157, see figure S1). During this period, the mean number of days on which at least one location was sent for an individual was 64 ± SD 25 (range 9–121). These two variables are highly correlated (Pearson's r=0.84), so we only included "period" in our models to control for the between-individual differences in overall time they were followed.

For each individual, we defined the following variables. (1) The number of home ranges (hereafter called residency areas). We identified residency areas with density-based spatial clustering using the R-package *tdbscan* (Valcu 2019). We set the following arbitrary parameters, determined based on visual inspection of the tracks (figure S2): 1 km as size of the epsilon neighbourhood (the set of locations within a specified radius around a given location); 10 as the minimum number of locations in the epsilon neighbourhood (core locations); 20 locations as maximum relative temporal lag (the number of locations in between two potential residency areas independent of the time passed in between); 60 hours as minimum time difference between the last and the first entry in the area. Residency areas were calculated as 90% home-range size with Kernel density estimation (UD) using the package *adehabitatHR* (Calenge 2011) (see figure S2 for examples). (2) Tenure in each residency area, defined as the period between the first and the last data point in this area. (3) The Euclidian distance between the nest and the last observed residency area (within the

200-day period). (4) The cumulative distance among residency areas (within the 200-day period). We tested if the different frequency of locations sent by individuals with the two transmitter types (Ecotone TI and Microwave TI) had an influence on the definition of our variables. As we found no significant effect, we used the data together.

We ran a linear mixed model for each of the four response variables with sex (factor), morph (continuous, scaled) and their second-order interaction as fixed effects. As the interaction term was not significant (see table 5.2) we did not retain it. We also included year (factor), number of days an individual was followed (continuous, scaled) and emigration date (Julian, scaled) as covariates and nest and pair identity as random effects. Tenure was normalized by log10-transformation and distances were normalized by square-root transformation before analyses. For the model that predicted tenure we included individual identity as additional random effect because we had repeated measures per individual.

For each residency area we calculated the proportion of forested habitat based on raster data from the CORINE Land Cover (EEA 2012) with a 100 m resolution grid. Proportion of forested habitat was derived for each area using packages *raster* (Hijmans et al. 2015), *sp* (Pebesma and Bivand 2005), and *rgdal* (Bivand et al. 2015). For analysis, we weighted this proportion by tenure as an estimate of overall individual presence in a given habitat. Weighted proportions were arcsine-squareroot transformed prior to analysis. We ran a linear mixed model with proportion of forested habitat (weighted) as response variable and morph, sex and year as fixed effects. We included nest and pair identity as random effects.

For all models, we confirmed that there was no collinearity among the predictors.

Results

Effects on emigration date

All 64 buzzards that survived until independence left their natal area, i.e. emigrated, in their first summer, with a peak in August in both years (range: 20 July–23 October, figure 5.1a). Emigration date correlated positively with hatching date and negatively with brood size, but was independent of plumage colour morph and sex (figure 5.1b, tables 5.1 and S1).

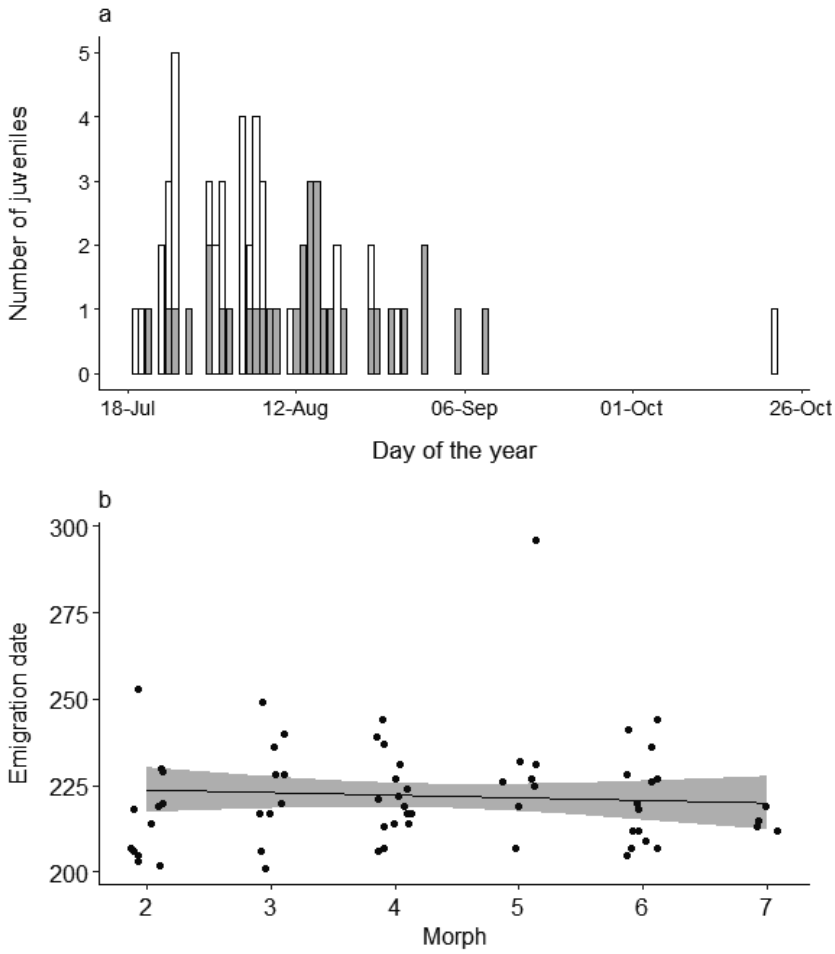


Figure 5.1: Emigration timing for 64 juvenile buzzards. **a**) The distribution of emigration dates for juveniles tagged in 2015 (white bars) and in 2016 (grey bars). **b**) Relationship between emigration date (Julian) and colour morph. Larger values represent lighter-coloured individuals. Shown are the regression line with 95% confidence interval (based on the model in table 5.1) and the raw data (points, jittered horizontally to avoid overlap).

Table 5.1: Results of a linear mixed model explaining variation in emigration date (Julian) of 64 juvenile buzzards in relation to sex (female as reference category) and morph (continuous, dark to very light, scaled). We controlled for year (2015 as reference category), hatching date (Julian, scaled), brood size (numeric, range 1–3) and body condition index (scaled) and included nest and pair identity as random effects. Shown are the results from the simplified model, except for the interaction term estimate, which is from the full model.

Predictors	**Estimate**	***CI***	***P***
Intercept	238.64	224.23 – 253.04	
Sex: male	-5.12	-12.17 – 1.93	0.16
Morph*	-1.18	-5.00 – 2.64	0.54
Sex: male × Morph*	-0.47	-7.90 – 6.96	0.90
Year: 2016	4.79	-2.52 – 12.09	0.20
Hatching date	5.53	1.47 – 9.59	**0.008**
Brood size	-7.23	-13.01 – -1.45	**0.014**
Body index	0.44	-3.06 – 3.95	0.80
Random Effects			
σ^2	185.03		
$\tau_{00\ nest}$	0.00		
$\tau_{00\ pair\ ID}$	1.82		
$ICC_{\ nest}$	0.00		
$ICC_{\ pair\ ID}$	0.01		
Observations	64		

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient
*on a seven-morph scale

Effects on dispersal behaviour

Individual buzzards had on average 6.2 (range 1–12) residency areas between fledging and the subsequent January. Darker buzzards visited significantly more residency areas in this period compared to lighter buzzards (figure 5.2a, tables 5.2 and S2). Tenure per residency area varied from 2.5 to 125.7 days (mean: 19.8 days ±25, n=396), and was unrelated to morph, or to any other explanatory variable. Dispersal distance varied widely within the population, with some individuals settling only <1 km from their natal nest, whereas 13 individuals moved over 100 km, mostly in southerly direction. Note that this could be viewed as seasonal migration, but due to the geography of the area, movements in northerly and westerly directions were restricted by the sea. Especially in 2015 many individuals settled in the winter in an area ca 30 km west of the natal area where an outbreak of common voles occurred (Bijlsma 2016). Dispersal distance was not correlated with colour morph or any other explanatory variable (figure 5.2c, table 5.2). The cumulative distance travelled varied from 0.02 - 635.5 km (mean: 132.9 ±139.7), and again, was unrelated to colour morph (figure 5.2d, table 5.2).

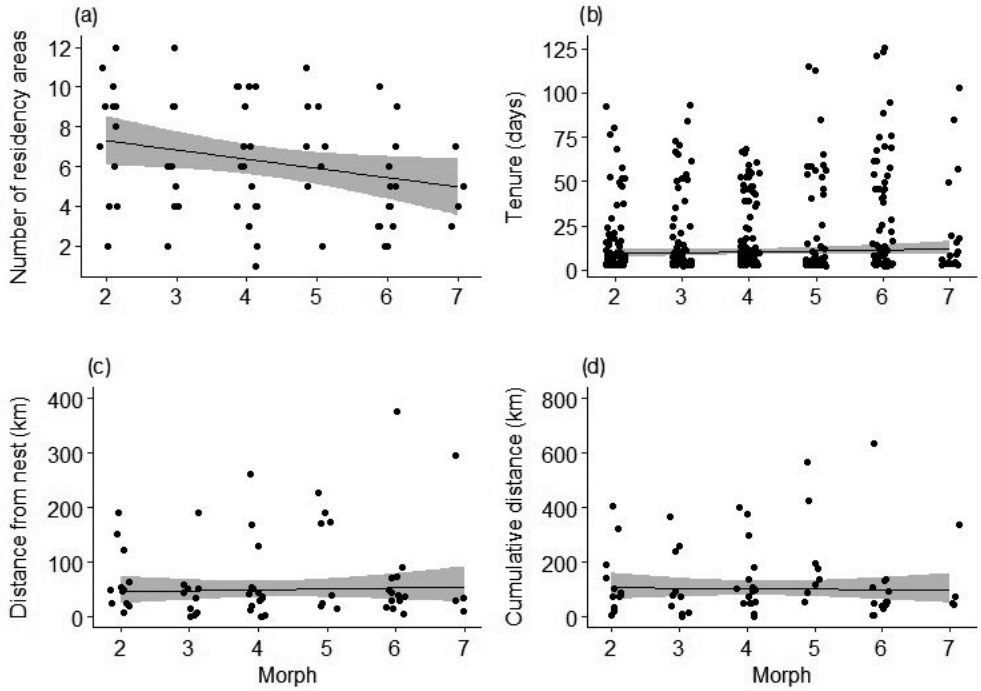


Figure 5.2: Relationship between aspects of natal dispersal behaviour and plumage colour morph in Common buzzards. Larger values on the X-axis represent lighter-coloured individuals. Shown are raw data (points, jittered horizontally to avoid overlap) and regression lines with 95% confidence intervals based on the back-transformed estimates from the models in table 5.2. **a**) number of residency areas visited, **b**) tenure in each residency area, **c**) dispersal distance and **d**) cumulative distance travelled.

					Random effects						
Dependent variable	***Predictors***	**Estimate**	***CI***	***P***	σ^2	$\tau_{00\ nest}$	$\tau_{00\ pair}$	$\tau_{00\ tag}$	ICC_{nest}	ICC_{pair}	ICC_{tag}
Residency areas	Intercept	6.67	5.42 – 7.92		4.80	0.00	1.50		0.00	0.24	
	Sex: male	0.64	-0.62 – 1.90	0.32							
	Morph*	-0.73	-1.41 – -0.05	**0.035**							
	Year: 2016	-1.55	-2.83 – -0.28	**0.017**							
	Recording days	0.91	0.24 – 1.57	**0.007**							
	Emigration date	-0.78	-1.42 – -0.13	**0.018**							
Tenure	Intercept	2.32	2.05 – 2.58		1.18	0.00	0.08	0.00	0.00	0.06	0.00
	Sex: male	-0.08	-0.34 – 0.18	0.54							
	Morph*	0.08	-0.07 – 0.22	0.29							
	Year: 2016	0.17	-0.11 – 0.44	0.24							
	Recording days	0.05	-0.09 – 0.18	0.48							
	Emigration date	0.13	-0.01 – 0.26	0.06							
Dispersal distance	Intercept	7.25	5.22 – 9.29		18.04	0.00	0.00		0.00	0.00	
	Sex: male	0.77	-1.42 – 2.96	0.50							
	Morph*	0.19	-0.87 – 1.26	0.72							
	Sex: male × Morph*	1.17	-0.97 – 3.32	0.28							
	Year: 2016	-1.31	-3.55 – 0.93	0.25							
	Recording days	0.97	-0.18 – 2.12	0.01							
	Emigration date	-0.20	-1.33 – 0.92	0.72							
Cumulative distance	Intercept	9.63	7.10 – 12.17		18.04	0.00	0.00		0.00	0.00	
	Sex: male	2.04	-0.70 – 4.78	0.14							
	Morph*	-0.14	-1.47 – 1.20	0.84							
	Sex: male × Morph*	1.23	-1.46 – 3.92	0.37							
	Year: 2016	-1.48	-4.29 – 1.32	0.30							
	Recording days	1.81	0.38 – 3.24	**0.013**							
	Emigration date	-1.04	-2.45 – 0.36	0.15							

σ^2 = Variance

τ_{00} = Ratio of population variance between groups

ICC = Intraclass Correlation Coefficient

*on a seven-morph scale

Table 5.2: Results of linear mixed effect models explaining variation in aspects of dispersal behaviour of 64 juvenile buzzards during their first autumn (up to 200 days after tagging) in relation to sex (female as reference category) and morph (continuous, dark to very light, scaled). We controlled for year (2015 as reference category), number of recording days (scaled) and emigration date (Julian, scaled). Dependent variables were number of residency areas visited, tenure in each residency area, dispersal distance from the natal nest and the cumulative distance travelled. Tenure in each of the residency areas was normalized by $\log_{10}$ transformation and then modelled with individual, nest and pair identity as random effects. Distances were normalized by square-root transformation and then modelled with nest and pair identity as random effects. Shown are results from the simplified models, except for the interaction term estimate, which is from the full models.

We found no tendency for morph-dependent habitat choice, however there was no substantial variation in how forested habitats were. Buzzards of different morphs were equally likely to visit residency areas with a lower or higher proportion of forested habitat during the first months of the wandering stage (figure 5.3, tables 5.3 and S6).

We found evidence for family resemblance in both the number of residency areas (table 5.2; Intraclass correlation coefficient on pair ID: 0.24) and especially in habitat choice (table 5.3; forest cover of residency areas: ICC_{pair} ID=0.61). In both cases these resemblances were on the pair-ID level rather than on the nest level. Given that siblings were never observed to move together (see supplement), this suggests innate movement strategies, or effects of early ontogeny unrelated to nestling condition or timing of independence.

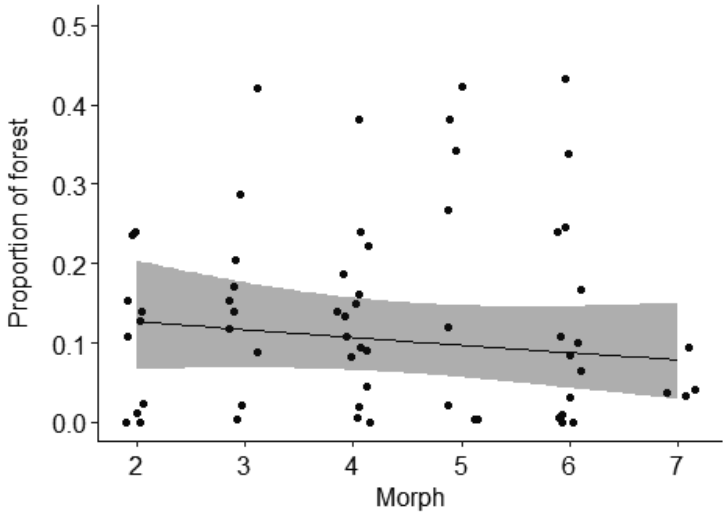


Figure 5.3: Relationship between the proportion of forested habitat in the residency areas visited by dispersing Common buzzards in relation to their plumage colour morph. Larger values on the X-axis represent lighter-coloured individuals. Shown are the regression line with 95% confidence interval based on the back-transformed model estimates (table 5.3) and the raw data (points, jittered horizontally to avoid overlap).

Table 5.3: Results of a linear mixed model explaining variation in the proportion of forested habitat in the residency areas visited by 64 juvenile buzzards in relation to plumage colour morph (continuous, dark to very light, scaled). We controlled for sex (female as reference category) and year (2015 as reference category) and included nest and pair identity as random effects. The proportion of forested habitat was weighted by tenure and then normalized by arcsine-square-root transformation.

Predictors		**Estimate**	***CI***	***P***
Intercept		0.28	0.18 – 0.38	
Sex: male		0.02	-0.05 – 0.10	0.57
Morph*		-0.03	-0.07 – 0.02	0.33
Year: 2016		0.07	-0.02 – 0.15	0.15
Random Effects				
σ^2	0.01			
$\tau_{00\ nest}$	0.00			
$\tau_{00\ pair\ ID}$	0.03			
ICC_{nest}	0.05			
$ICC_{pair\ ID}$	0.61			
Observations	64			

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient
*on a seven-morph scale

Discussion

To better understand the maintenance of colour polymorphisms, we tracked 64 juvenile Common buzzards from independence to five months into the wandering stage. We found that morphs only differed in the number of areas visited, but melanic coloration was not associated with other traits such as emigration timing, distance travelled and proportion of forested habitat chosen. We found that darker buzzards visited a higher number of residency areas during the first months of their wandering stage compared to lighter individuals. Our data are to some extent consistent with a German study, showing that dark juveniles were resighted further from their natal nest until November (Chakarov et al. 2013). As coloration and personality traits are associated in many species (Ducrest et al. 2008; Schweitzer et al. 2015) including Common buzzards (Boerner & Kruger, 2009), one way of interpreting this result is that darker buzzards are the proactive behavioural type and thus tend to be more exploratory than lighter individuals. Interindividual variation in melanic coloration is strongly heritable in this population (h^2=0.82; Kappers et al. 2018) and we showed that also one aspect of dispersal behaviour and habitat choice had intermediate to high family resemblance. This suggests that these traits may also be partially heritable in our population as it is in other species (e.g. Dingemanse et al 2002; Duckworth & Kruuk). Although we have not investigated genetic correlations between traits, our data support the notion that colouration and (spatial) behaviour might be linked as part of a behavioural syndrome.

We hypothesized that phenotype-habitat matching during this wandering phase could have been adaptive, as suggested in some raptors by the relationship between polymorphism, activity patterns and vegetation cover or moon light regime (Tate et al. 2016; San-Jose et al. 2019). These patterns suggested that variation in coloration might function as an adaptation driven by light conditions to exploit varying niches. Tate and colleagues (2016) found that breeding individuals of the dark morph in the Black sparrowhawks provided more prey in lower light conditions whereas individuals of the light morph provided more prey in brighter conditions. Similarly, San-Jose and colleagues (2019) showed that the red morph of Barn owls had a lower food intake in moon-lit nights than during new moons, whereas the white morph was unaffected by the moon cycle. Although habitat selection was not random (as shown by the high family resemblance), we did not find that darker morphs occupied the more forested areas. If indeed dark morphs would perform better in forested areas (as in the Black sparrowhawks), our results of more residency areas for darker individuals might be due to darker young birds experiencing more competition from breeding buzzards as they aim at settling in forested areas, where the density of territories is highest (Sovon 2018). Hence, the lack of habitat matching as observed in our data might be due to competitive exclusion by territorial pairs, rather than a lack of preference for these habitats. As aggression is more intense between conspecifics with similar colour morphs (Boerner & Krueger, 2009), this could cause frequency-dependent habitat selection and such a process will work against a process of phenotype-habitat matching.

Interestingly, we found that part of the variation in the proportion of forested habitat in residency areas was explained by between-pair differences (table 5.3). The ten

pairs from which we tagged juveniles in both years were breeding in the same territory as the year before, if not in the same tree. Thus, in our study we cannot disentangle if there is a heritability component in habitat choice or imprinting on the natal site.

Morphs did not differ in the timing of emigration. The juveniles tagged in our study emigrated all in one wave in their first summer, with no individuals emigrating after the first winter, as found in a French, a German and a British population (Nore and Malafosse 1992; Chakarov et al., 2013; Walls and Kenward 1997). Why these apparent population differences exist remains unknown, but the high density in our population (1.25 pairs/km2, see Materials and methods) may force individuals to leave soon after independence. Emigration occurred in directions most likely influenced by prey availability and the presence of adults. Indeed, in 2015 there was a strong tendency for juveniles to join groups of non-territorial buzzards in open fields in Friesland, near the end of a vole-plague (Bijlsma 2016).

Our study focussed on the association between colour morph and natal dispersal, because despite a valid theoretical background only few empirical studies tested this association. Previous studies about morph differences in dispersal considered the binary variable “dispersed or not” or the distance between the natal site and the site of the first breeding attempt within the study population or colony (Emaresi et al. 2014; Saino et al. 2014; Sumasgutner et al. 2016; Camacho 2018; Gangoso and Figuerola 2019). Tracking individuals from independence enabled us to investigate the relationship between melanic coloration and the wandering stage of natal dispersal, when juveniles sample and temporary settle in one or more residency areas before first breeding. In the first months after emigration, we found some evidence for an association between colour morph and one of the dispersal traits (number of residency areas visited). It would be of great interest to investigate the entire wandering stage until first breeding, which can last three to four years after emigration in Common buzzards (Dare 2015). Dispersal is an important yet poorly understood life-history stage and further investigating if and how morphs differ in dispersal behaviour during the wandering stage can improve our understanding of the ecological and social selection pressures acting on colour morphs, and answer whether morph frequencies are maintained over time or show directional changes in different breeding populations (Mueller et al. 2016, Kappers et al. 2020).

Acknowledgements

We thank the landowners for permission to work on their property. Special thanks to Raymond Klaassen who was our mentor on the application and use of the transmitters. Thanks to all those who assisted in the field, especially Rob Bijlsma, Andrea Wittenzellner, Agnes Türk, Wender Bil, Jorian Huisman.

Ethics

Tracking was approved by the ethical committee of the University of Groningen, The Netherlands (permit 7003A).

Data accessibility

Data are available from the Open Science Framework at https://osf.io/v5knm/.

Supplemental Material

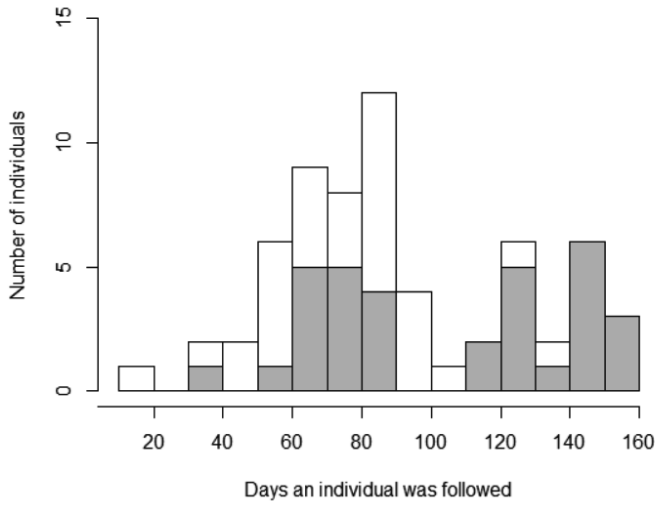
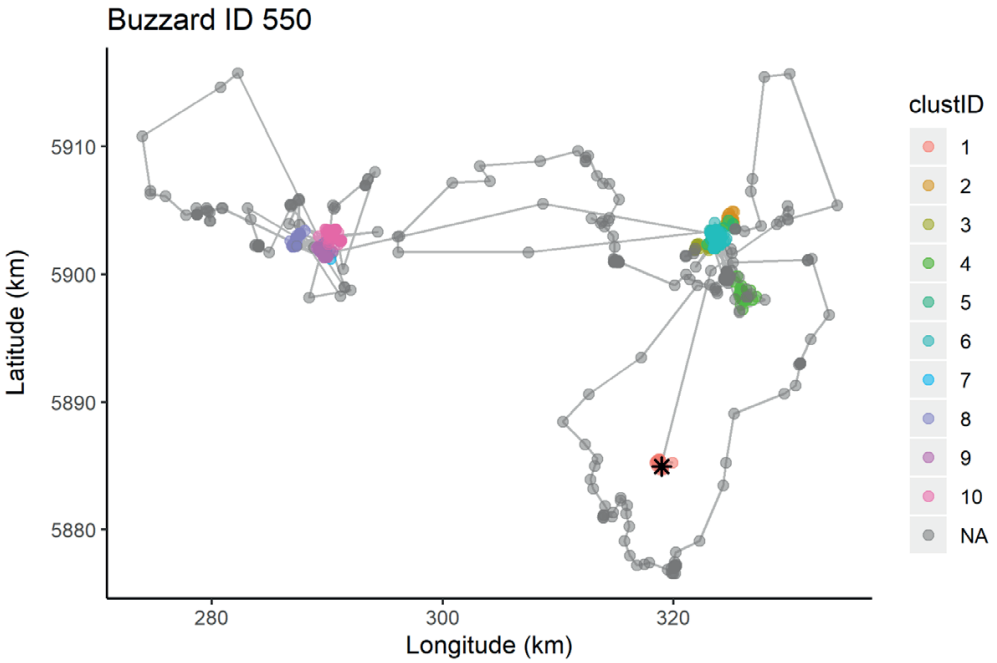
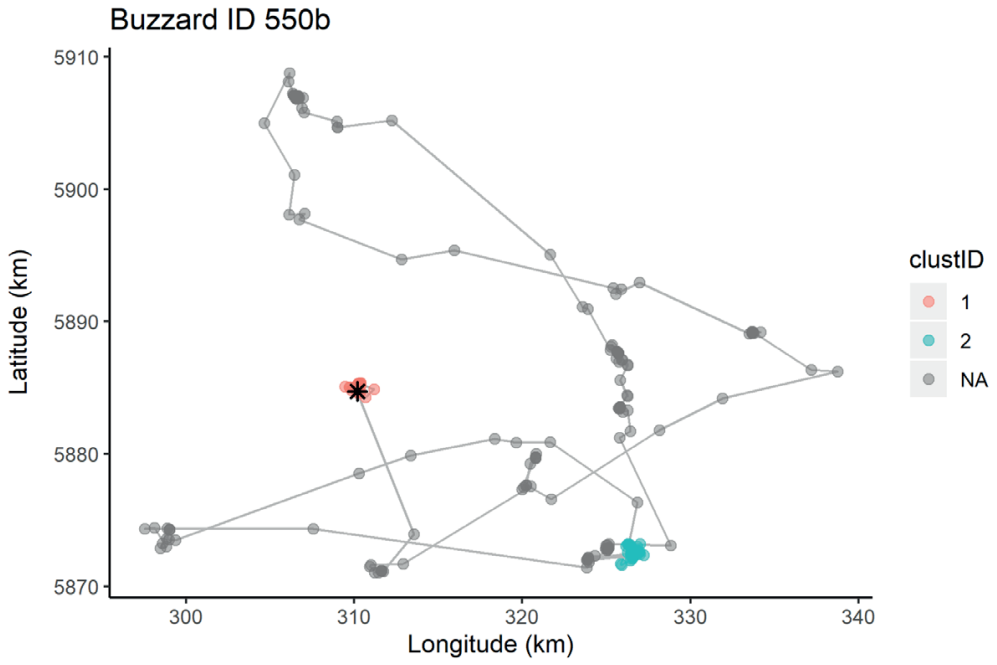


Figure S1: Total number of days an individual was followed after emigration and within the first 200 days after tagging (n=64). White bars represent individuals that were lost within the 200-days period and grey bars represent individuals that we could still follow after the 200-days period, even after a gap due to low battery level (see Materials and methods).

Figure S2: Tracks of all buzzards within 200 days after tagging. Grey points represent locations that do not belong to any residency area. Coloured points identify residency areas visited by buzzards and numbered according to the order of visit. Black asterisc identifies the location of the natal nest. See table S7 for more information on the buzzard ID.

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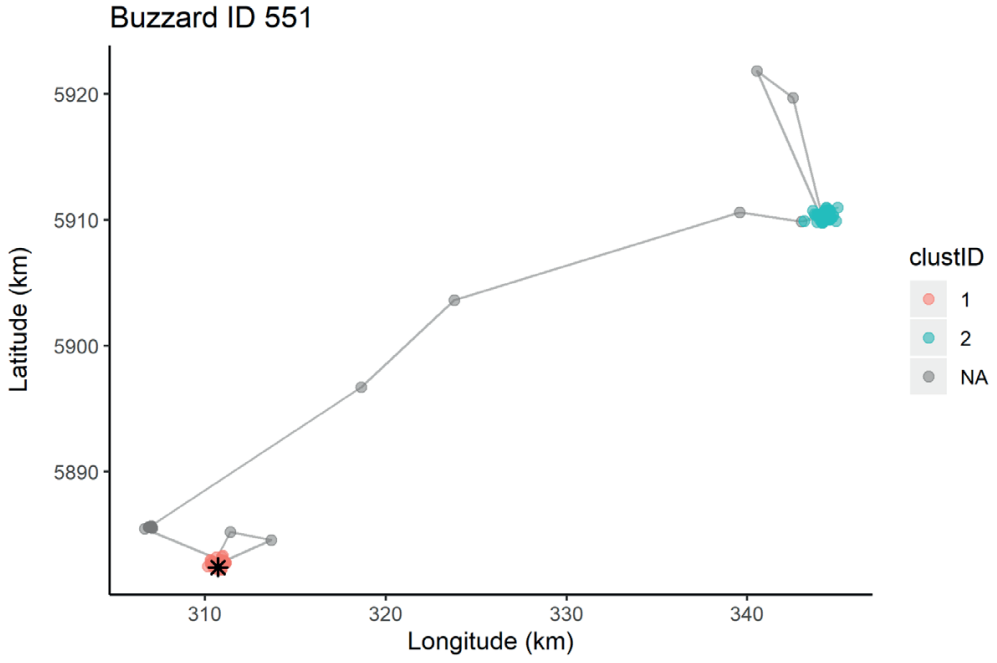
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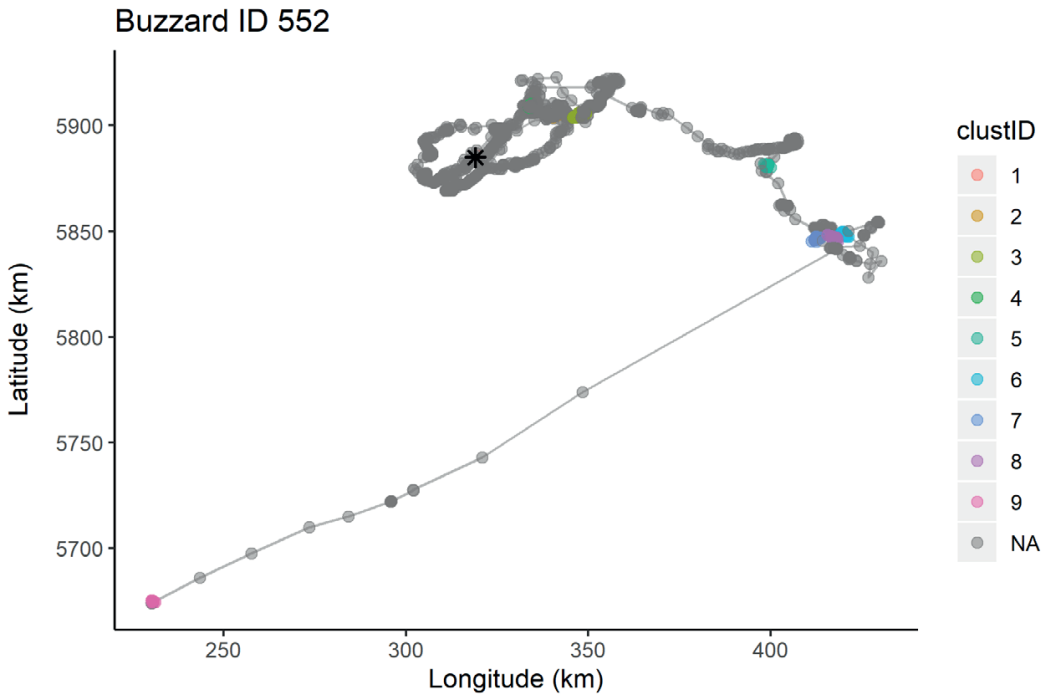
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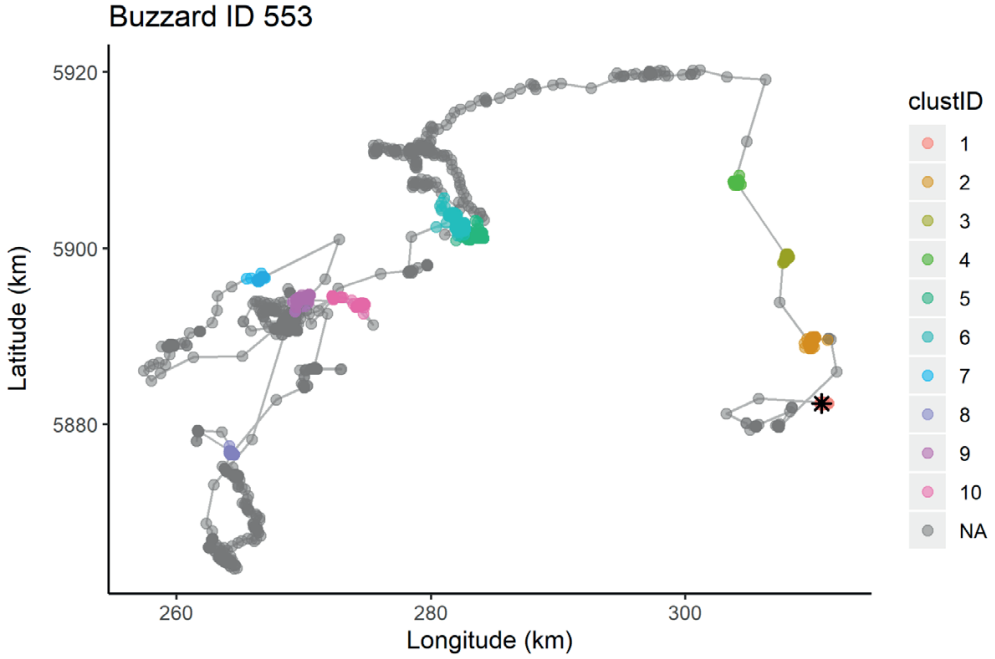
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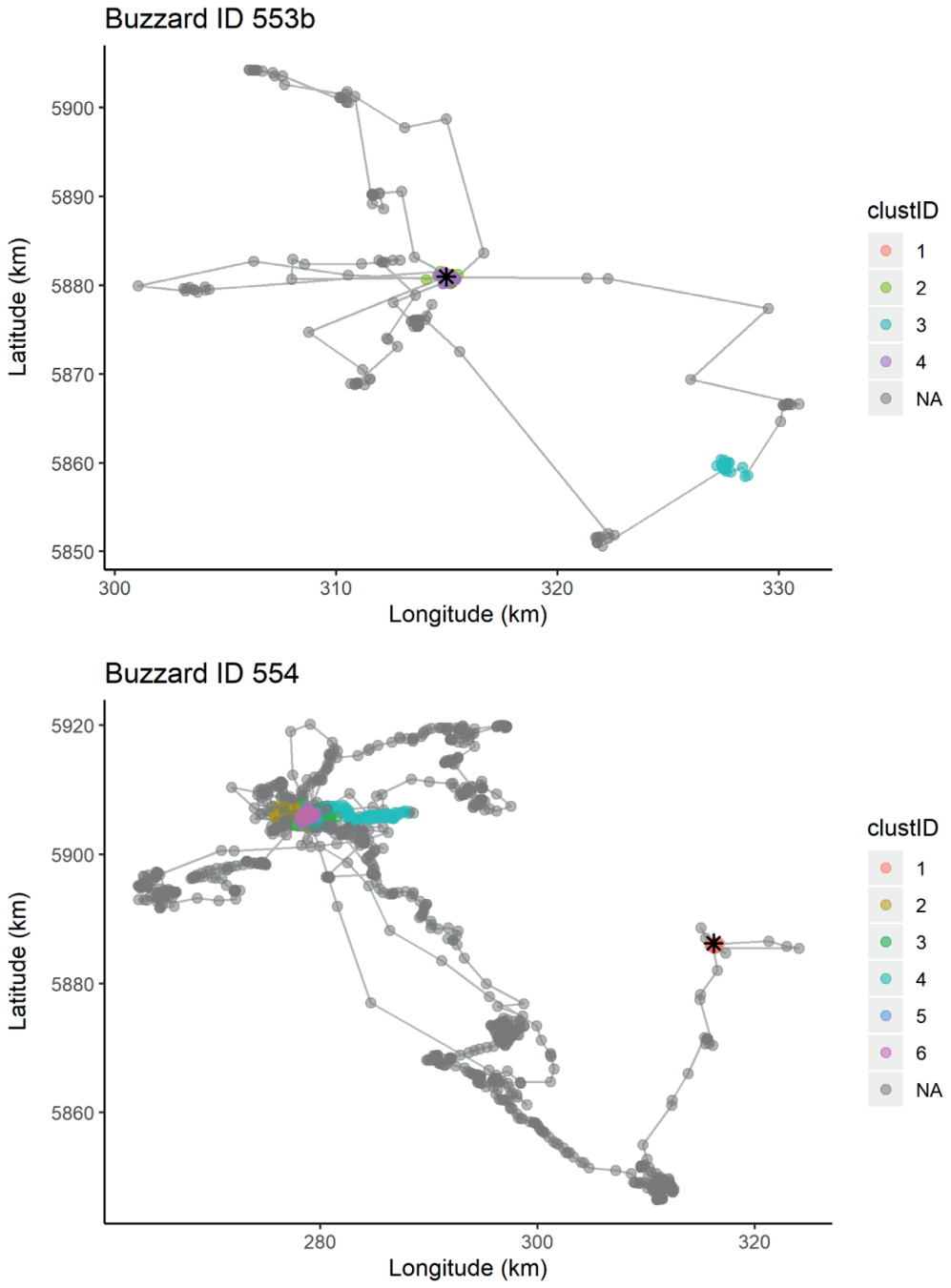
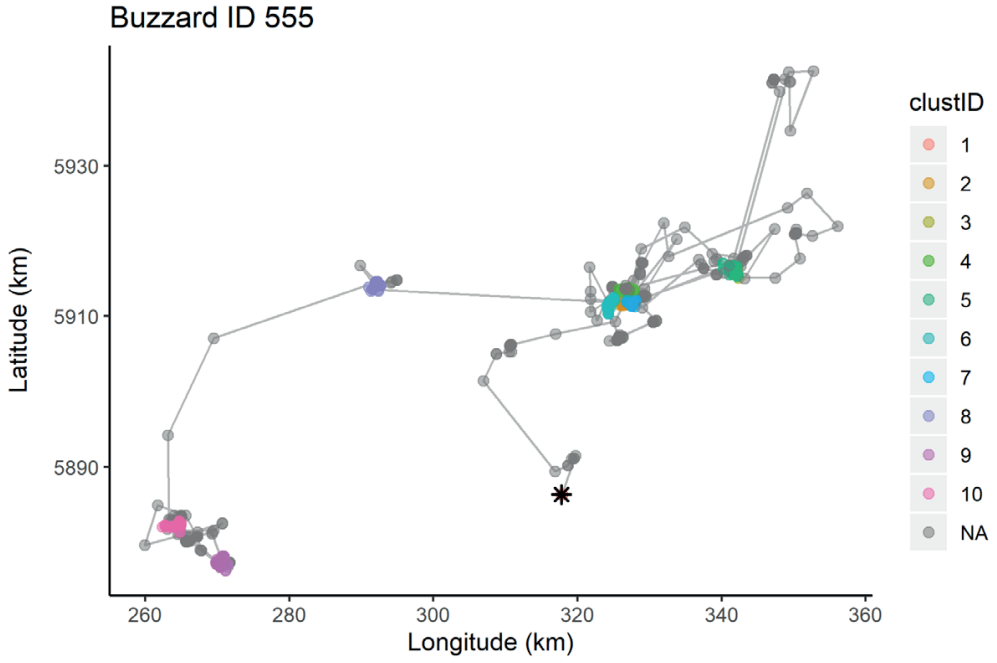


Figure S2: continued.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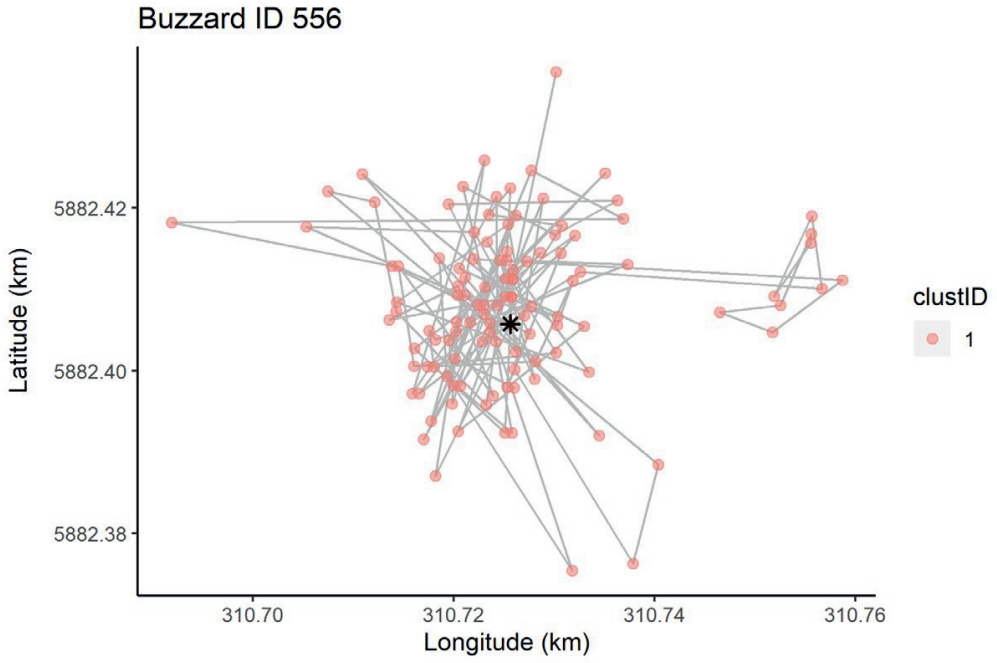
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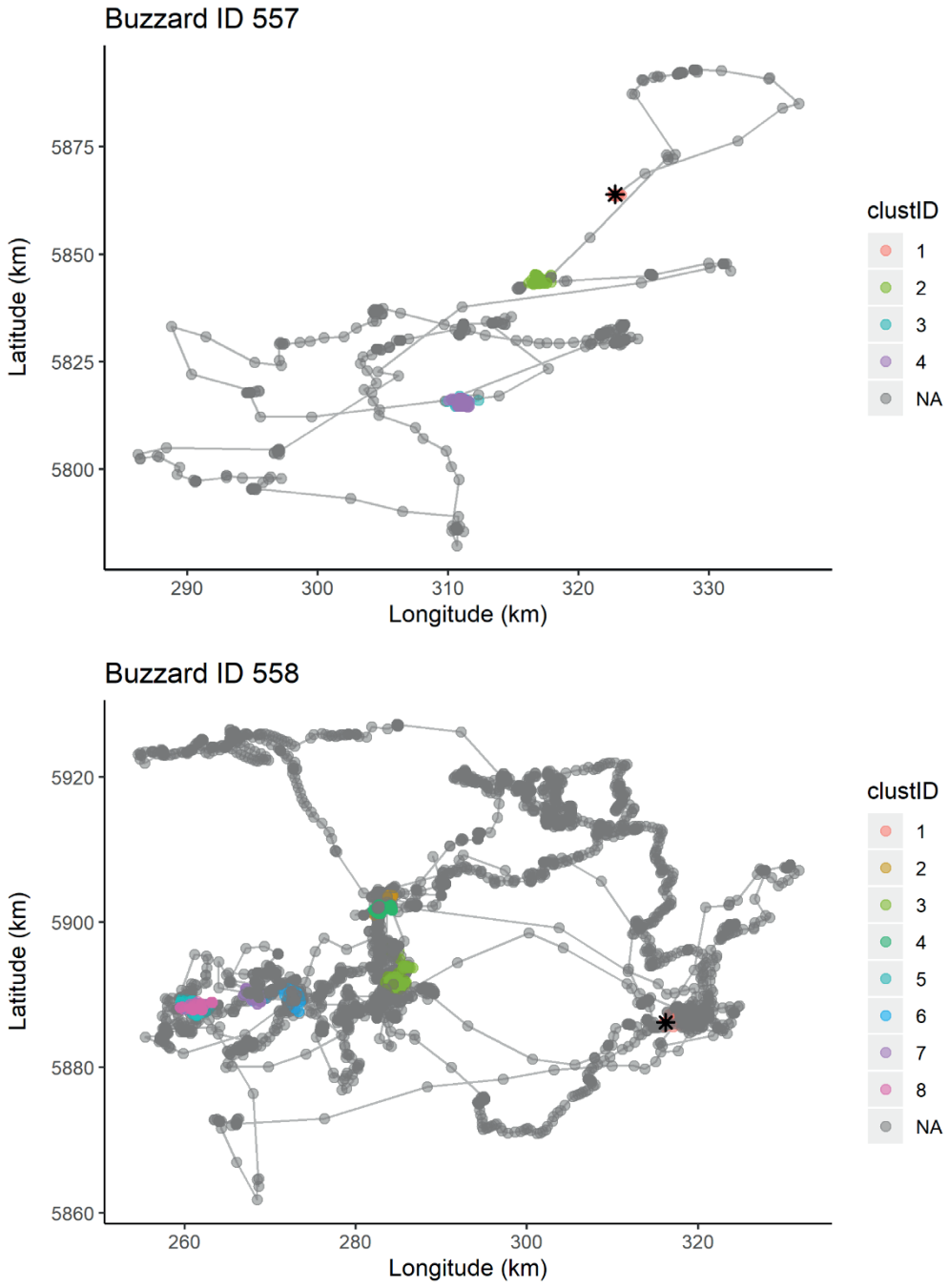
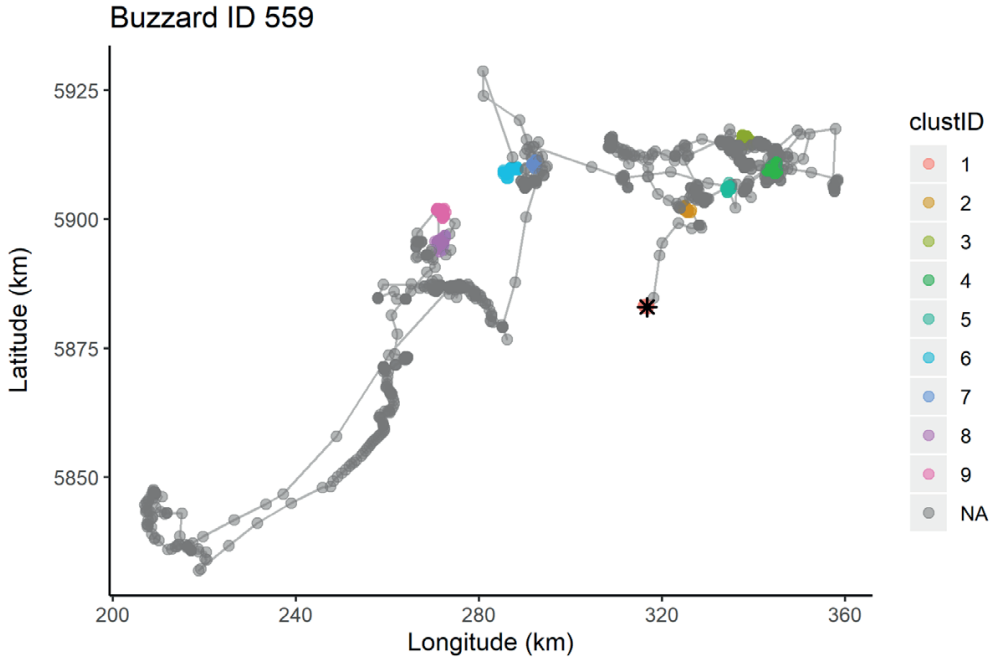


Figure S2: continued.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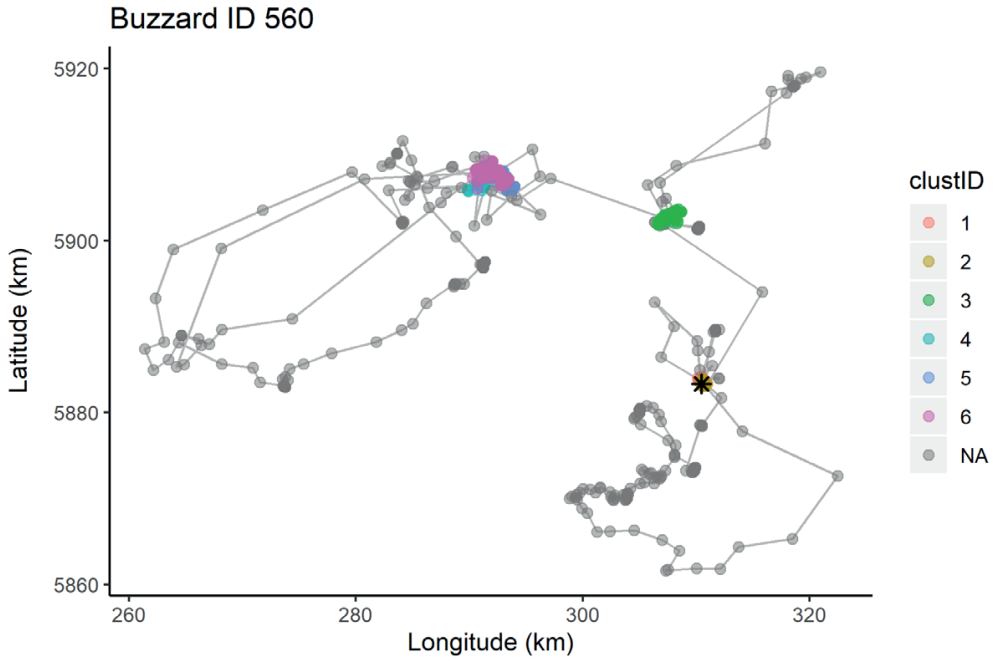
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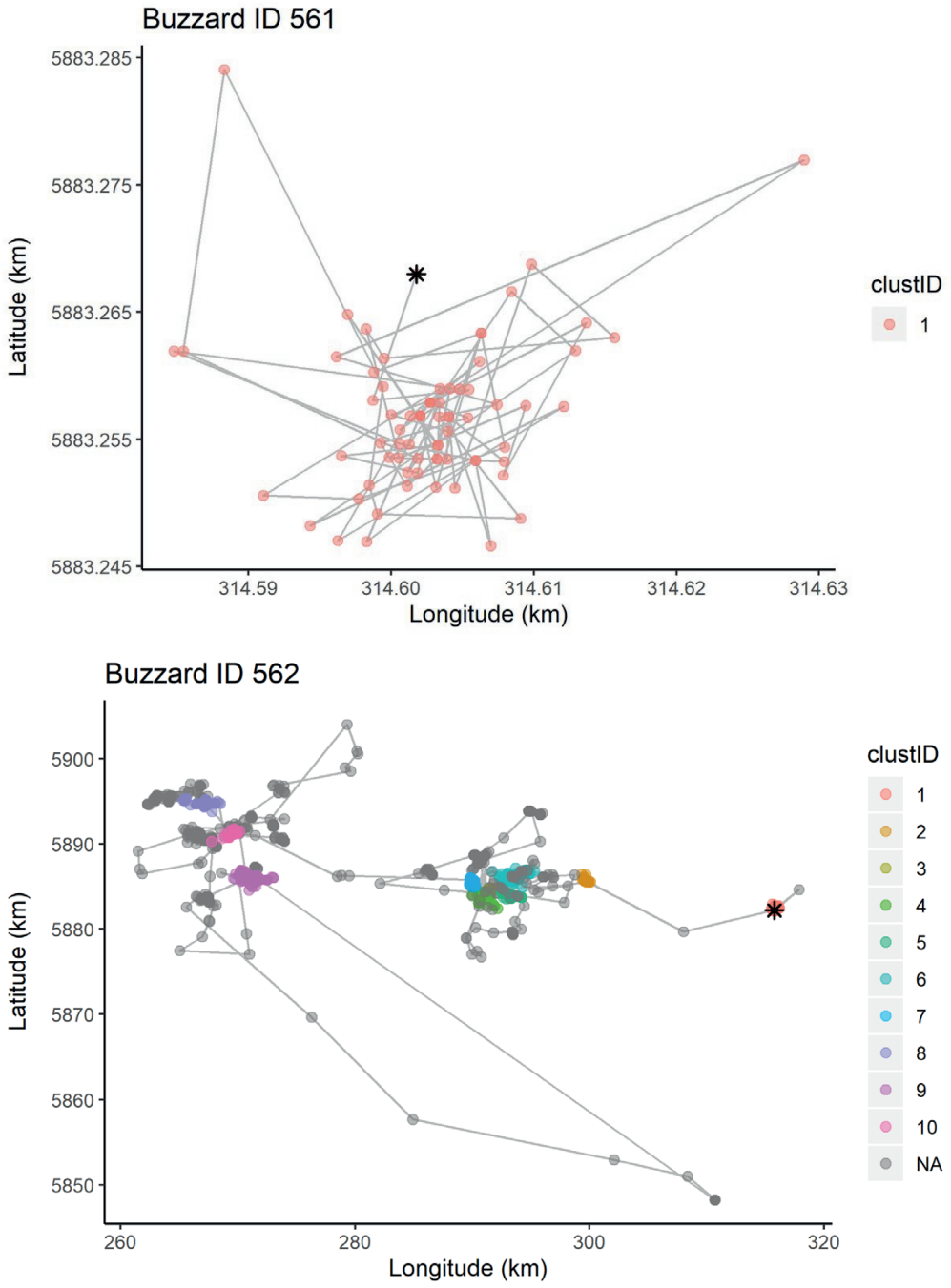
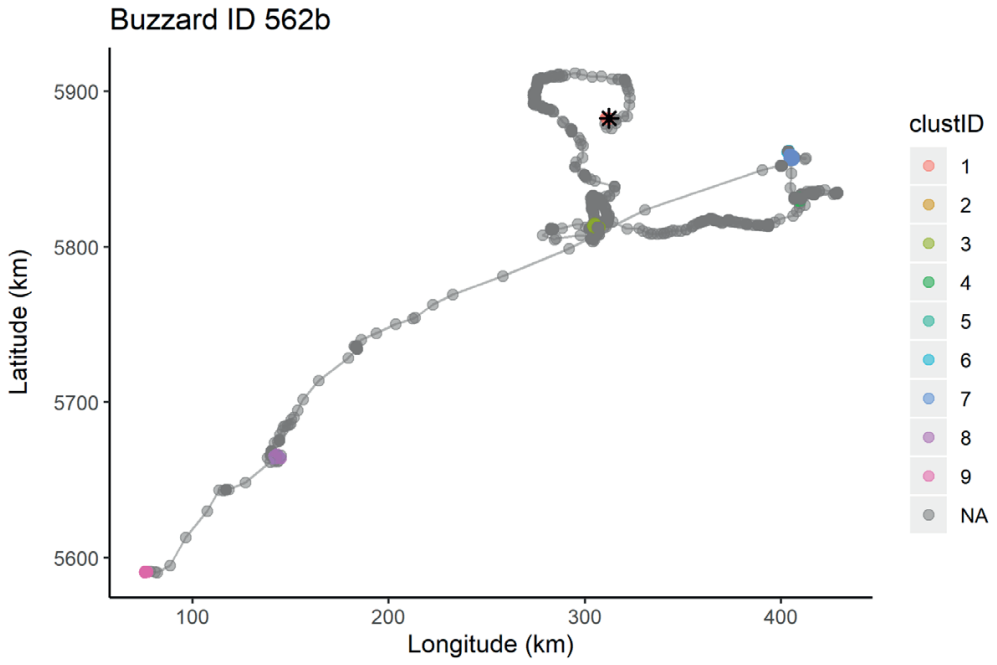


Figure S2: continued.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Latitude (km)
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Longitude (km)
clustID
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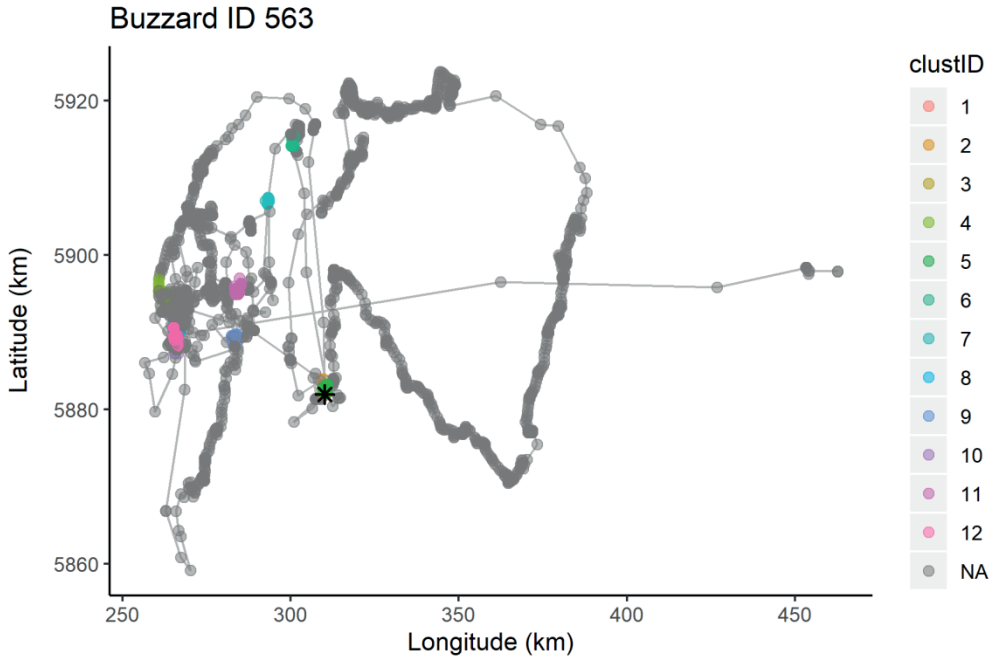
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5860
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Longitude (km)
clustID
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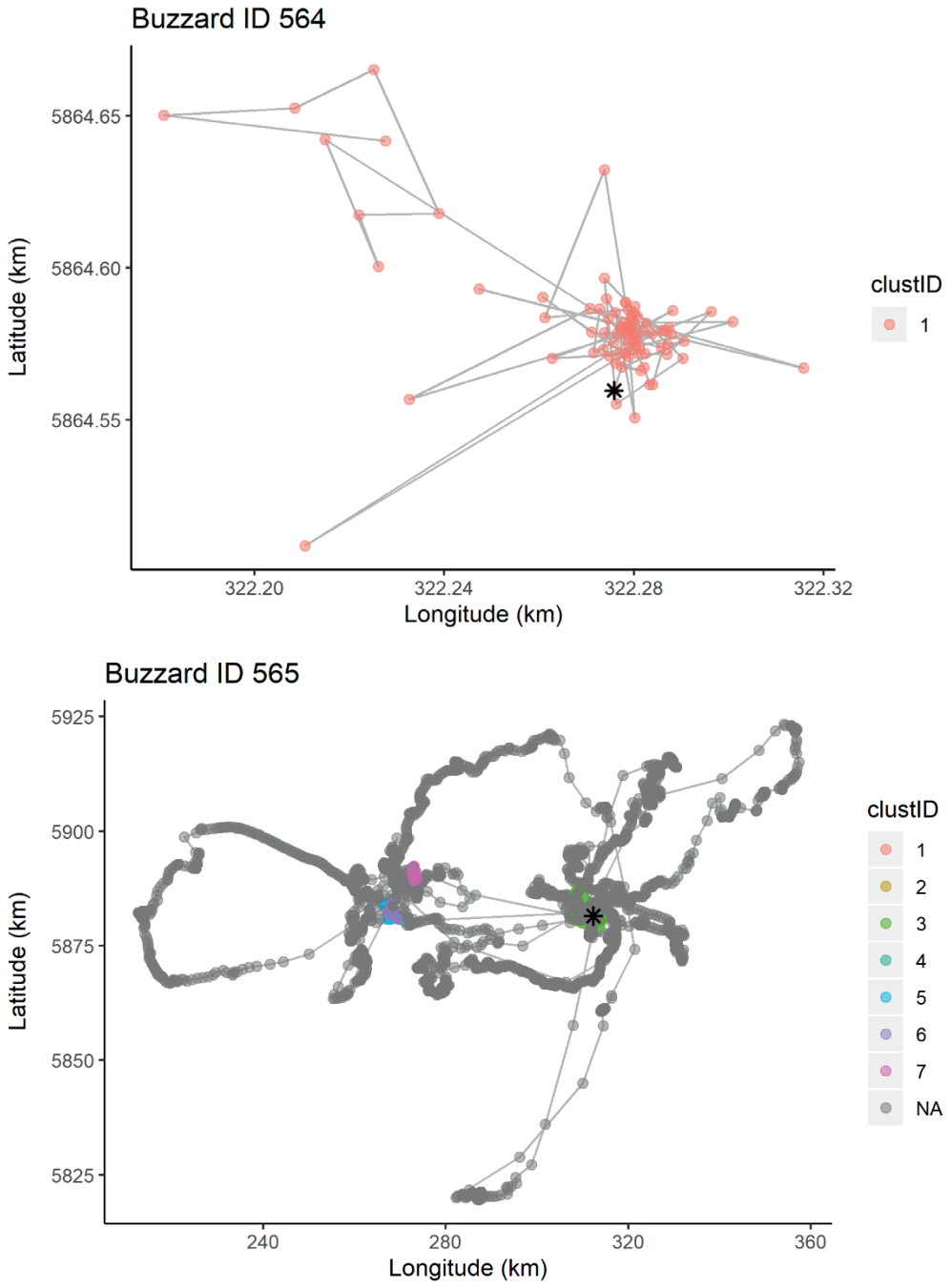

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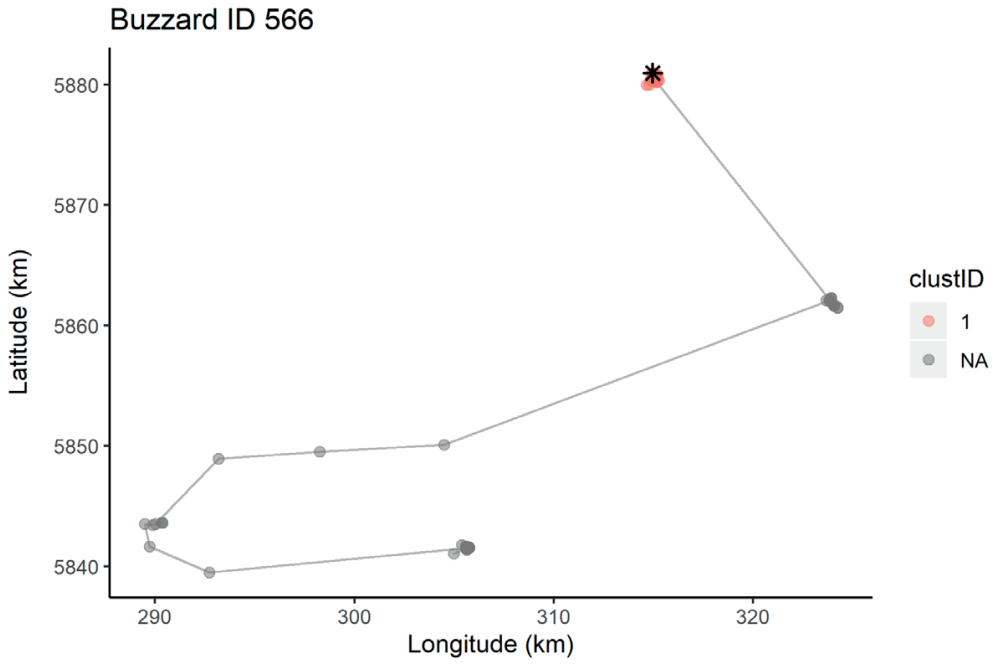
Buzzard ID 566
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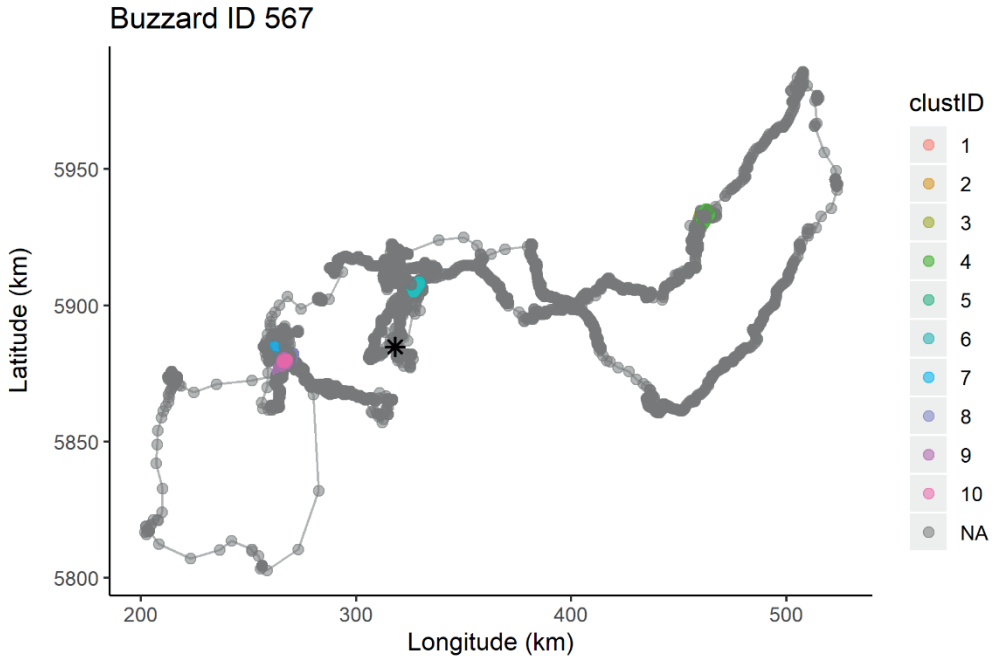
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Longitude (km)
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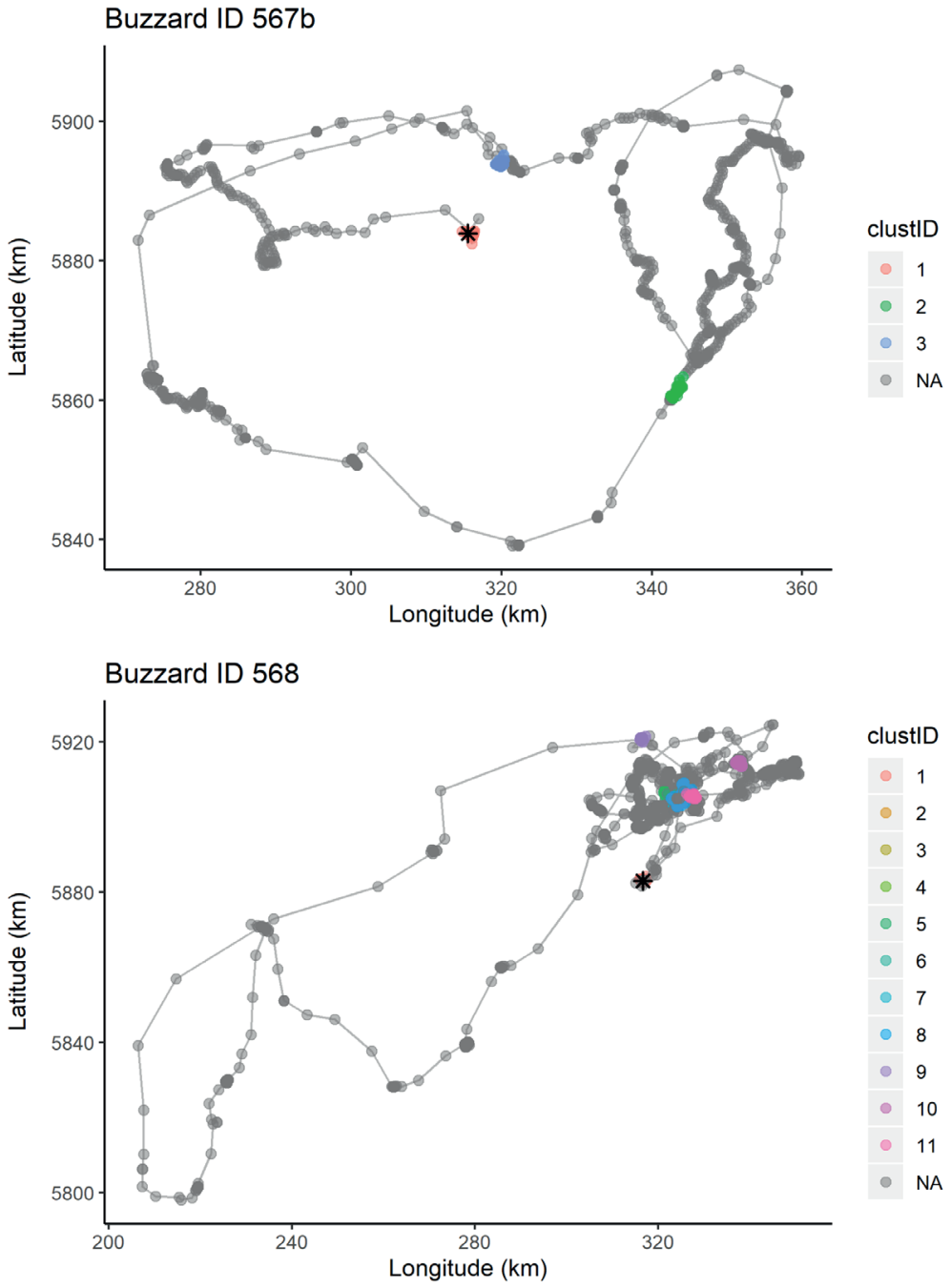

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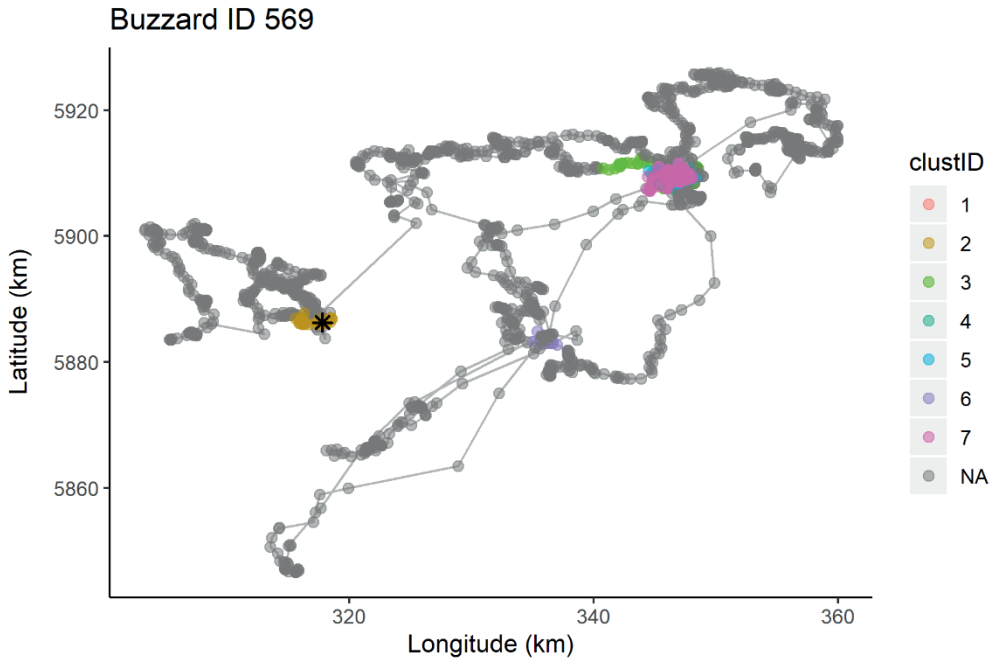
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Longitude (km)
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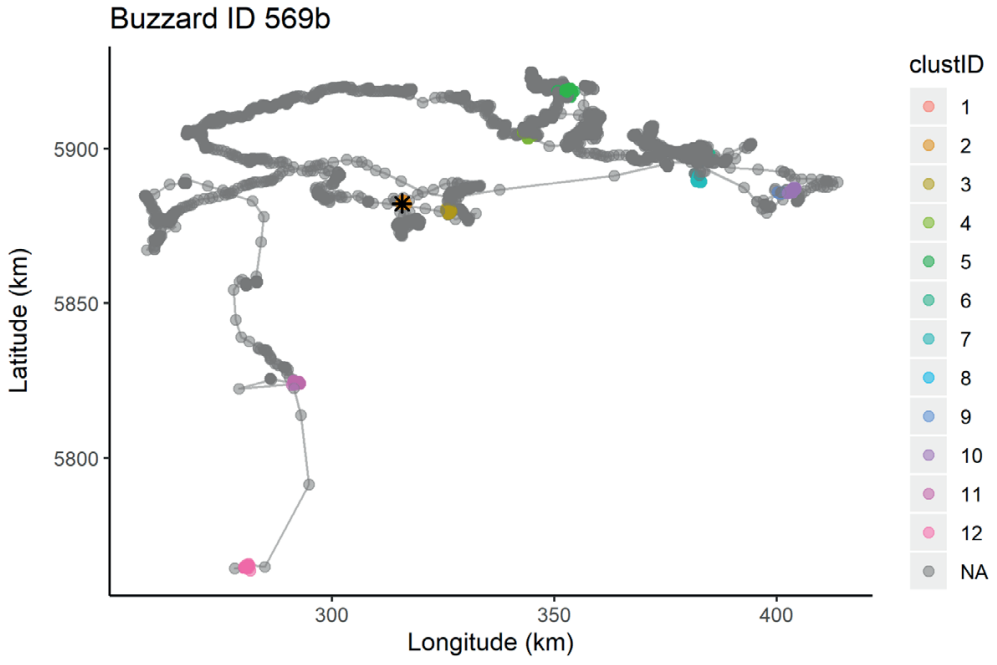
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Longitude (km)
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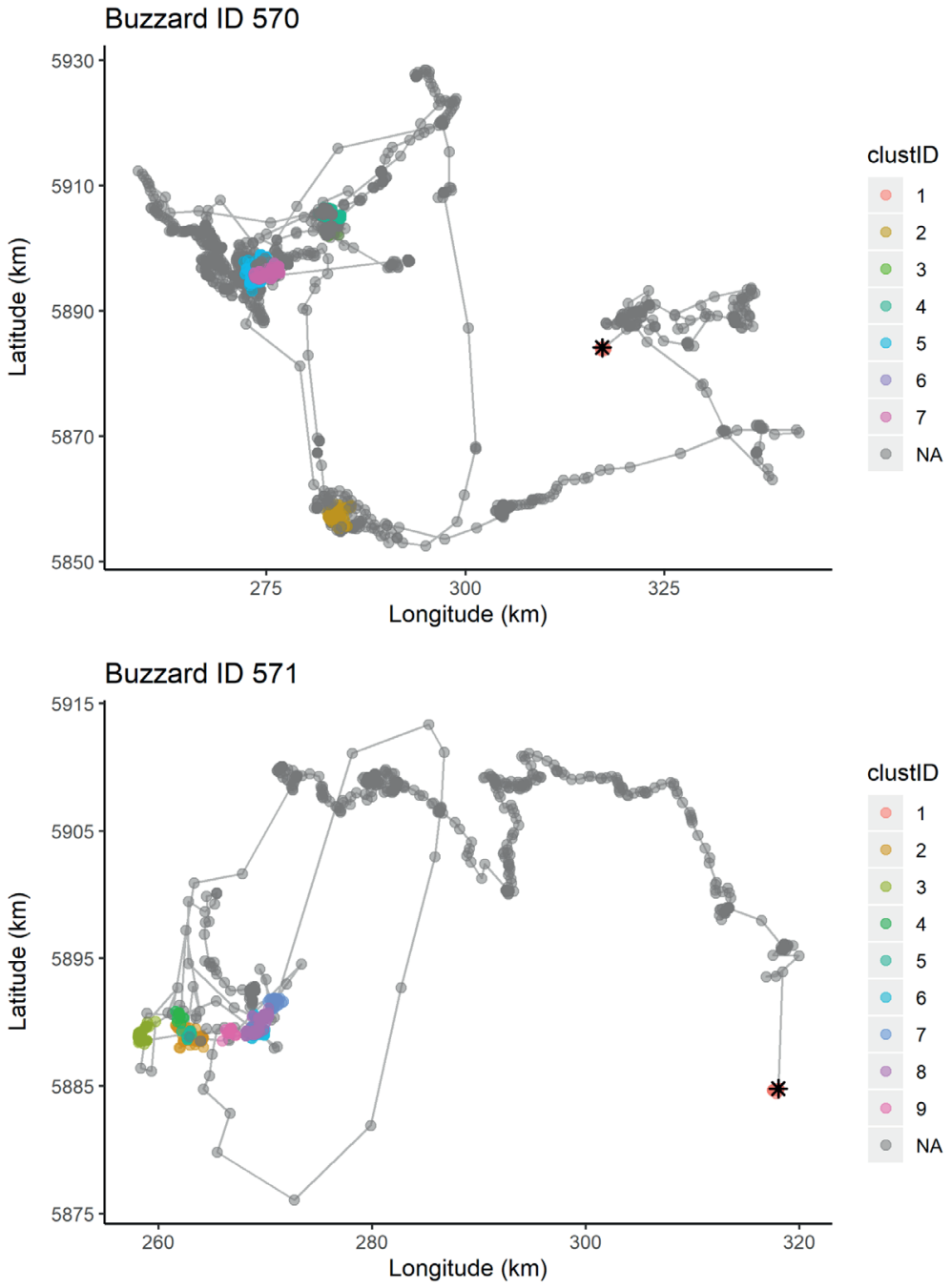

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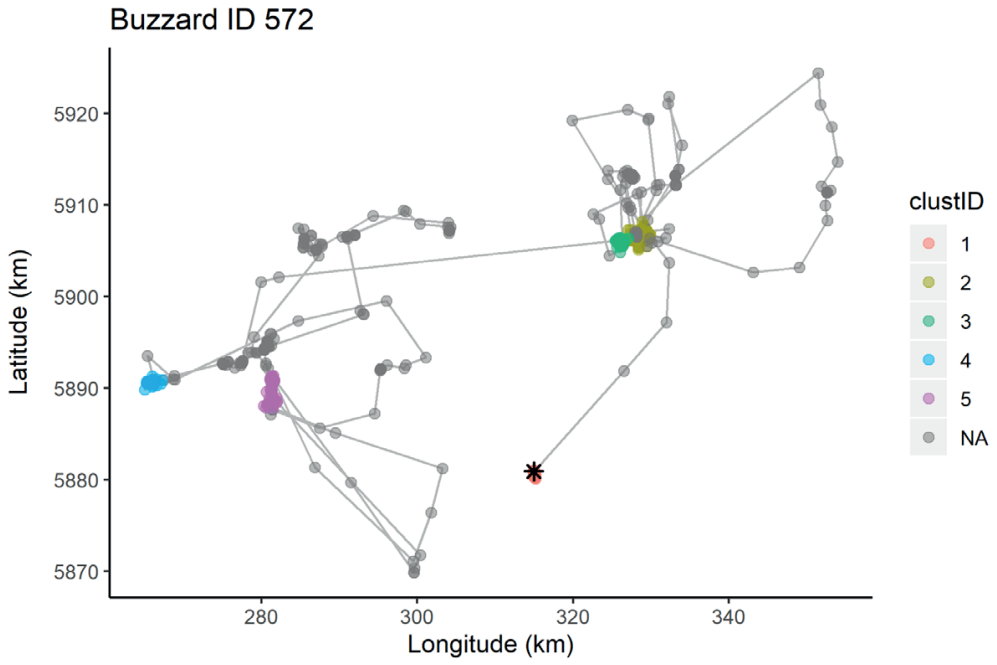
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340
Longitude (km)
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3
4
5
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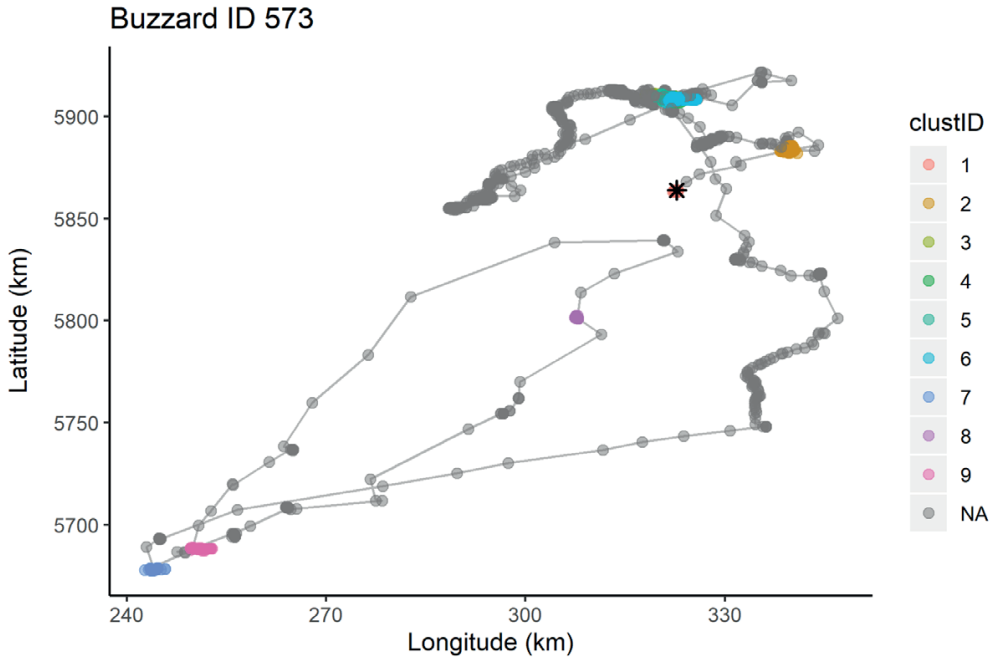
Buzzard ID 573
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5750
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270
300
330
Longitude (km)
clustID
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7
8
9
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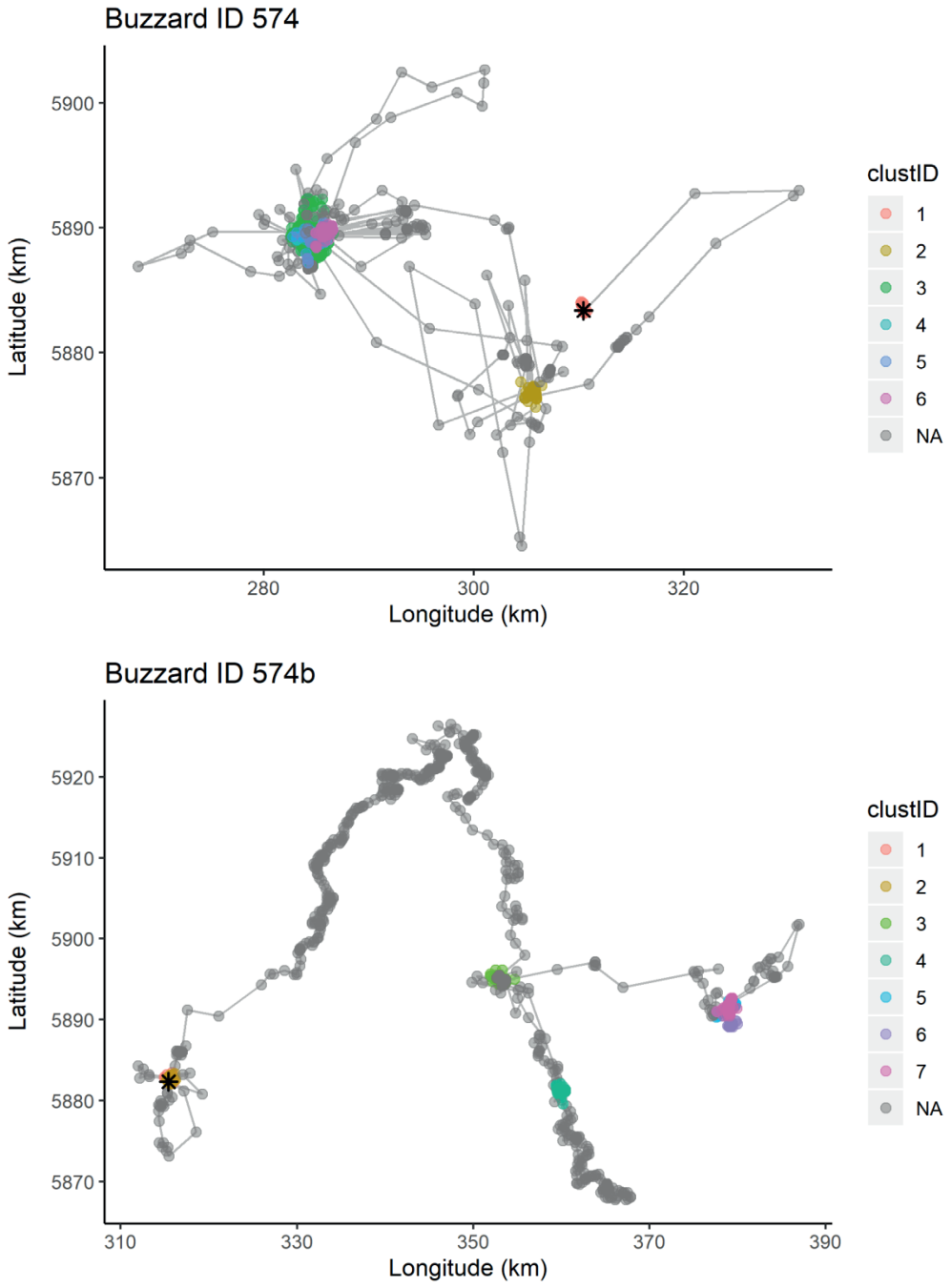
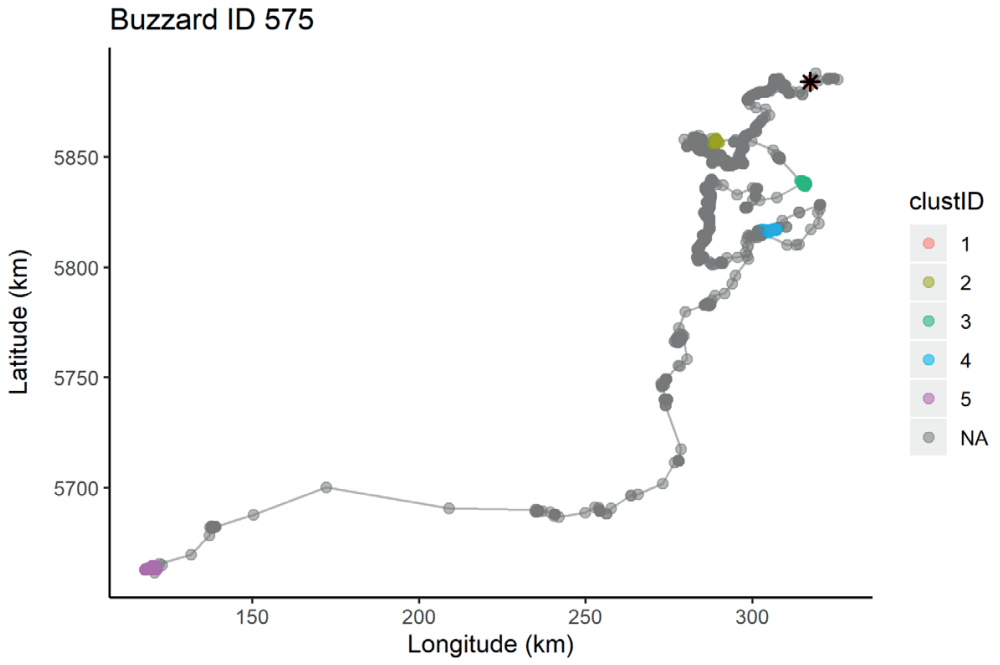


Figure S2: continued.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Longitude (km)
clustID
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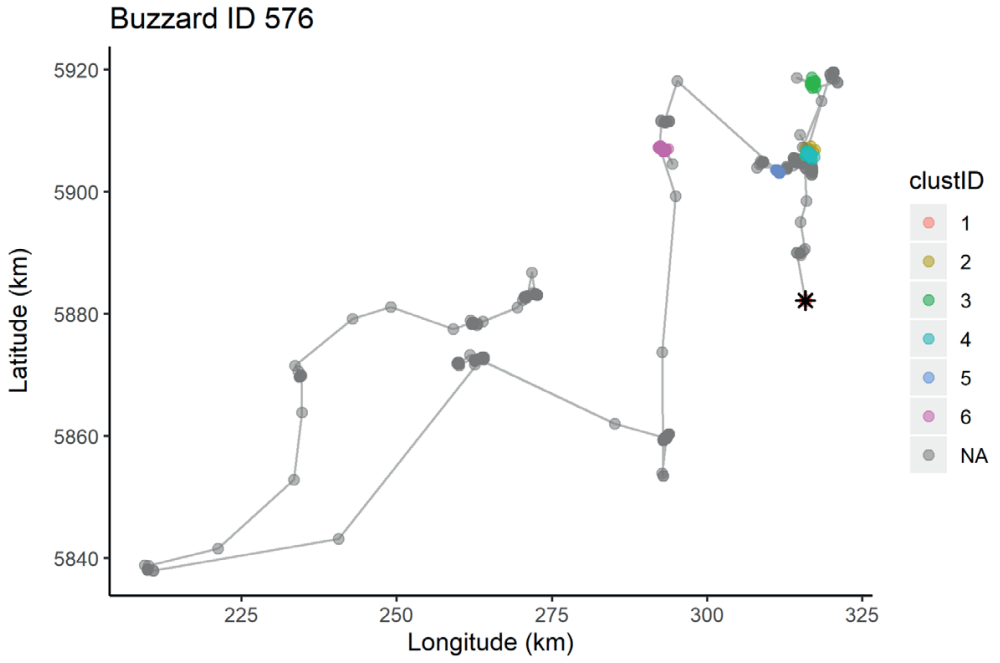
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325
Longitude (km)
clustID
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6
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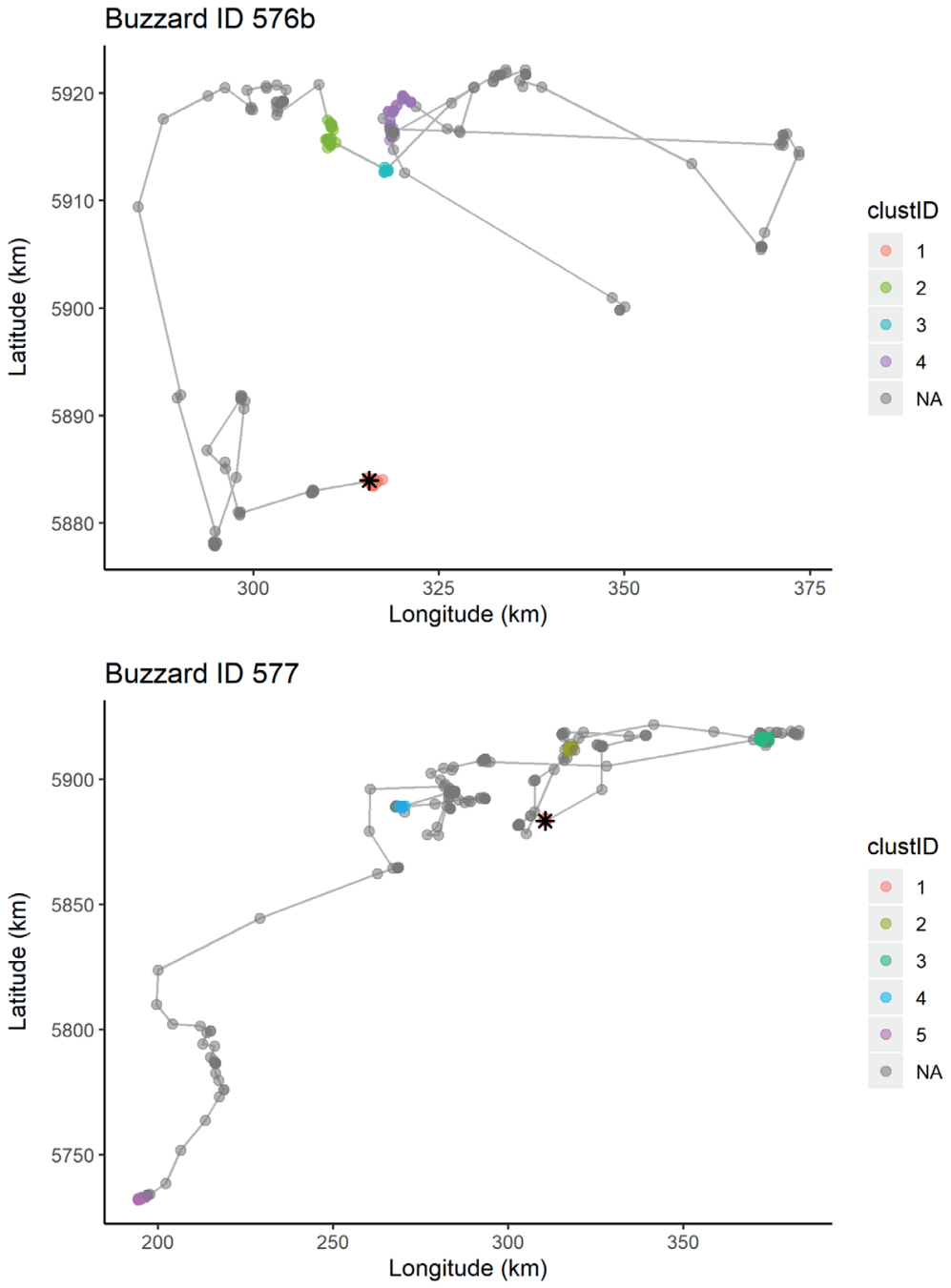

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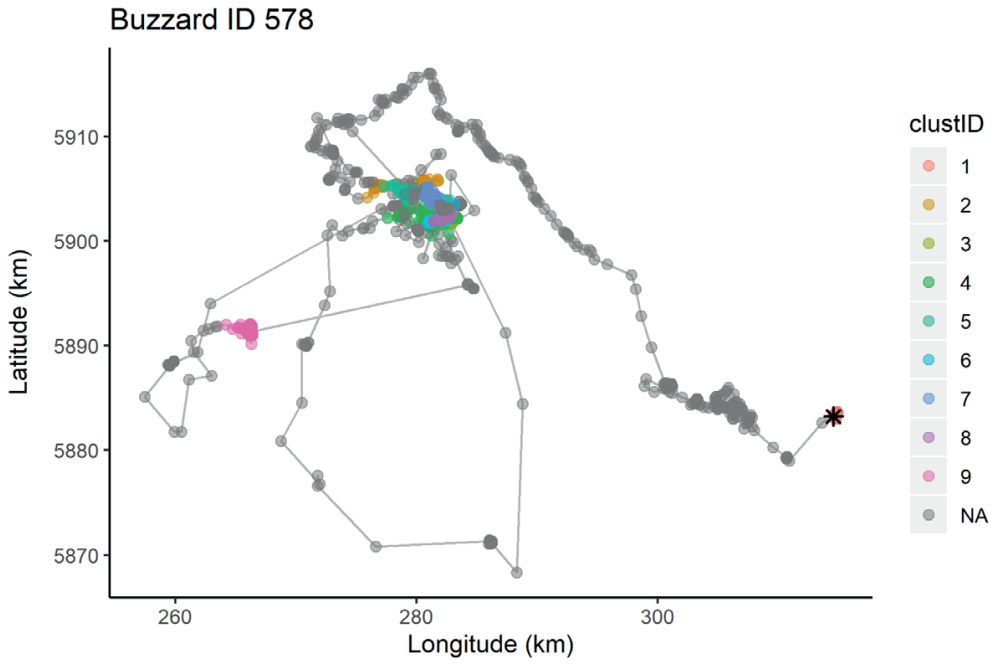
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Longitude (km)
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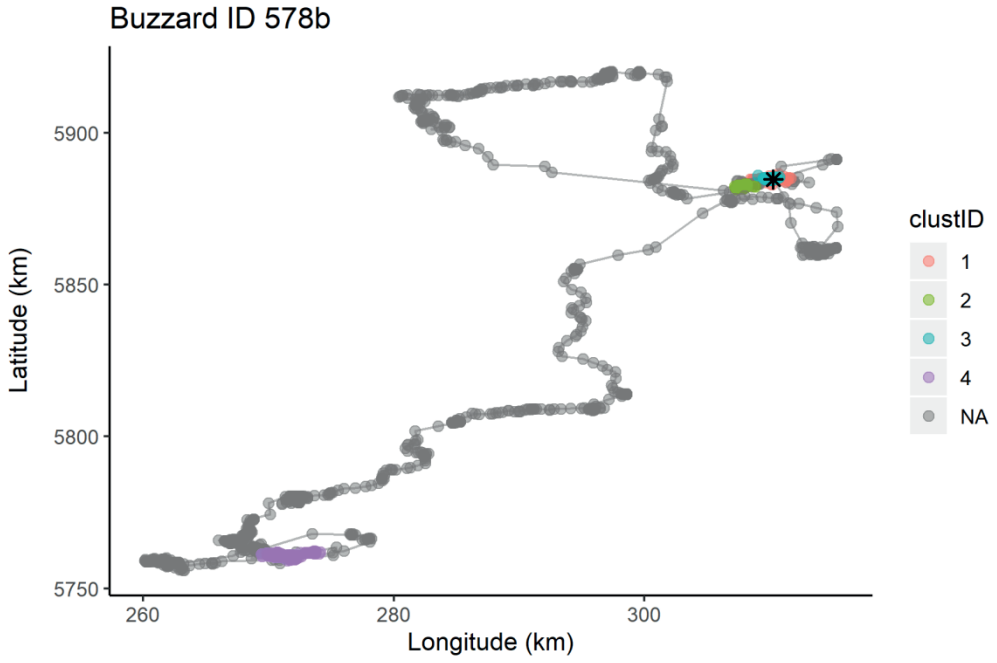
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300
Longitude (km)
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3
4
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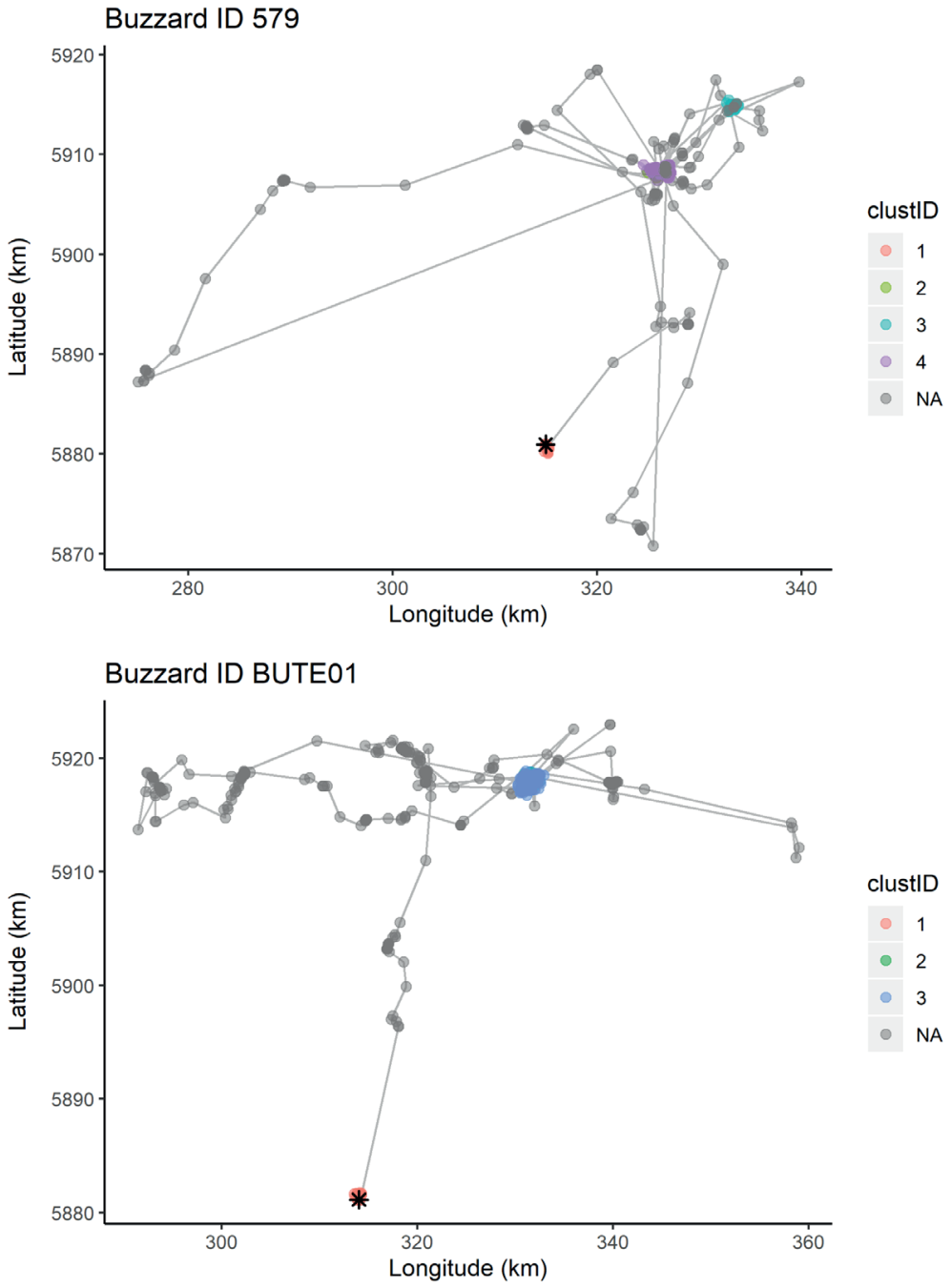
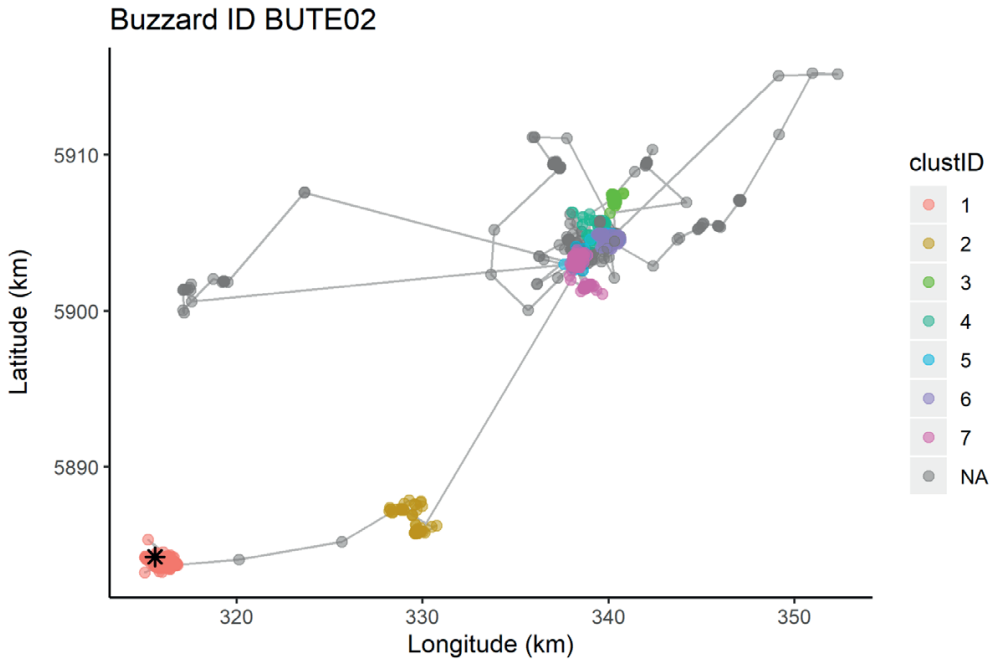


Figure S2: continued.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Longitude (km)
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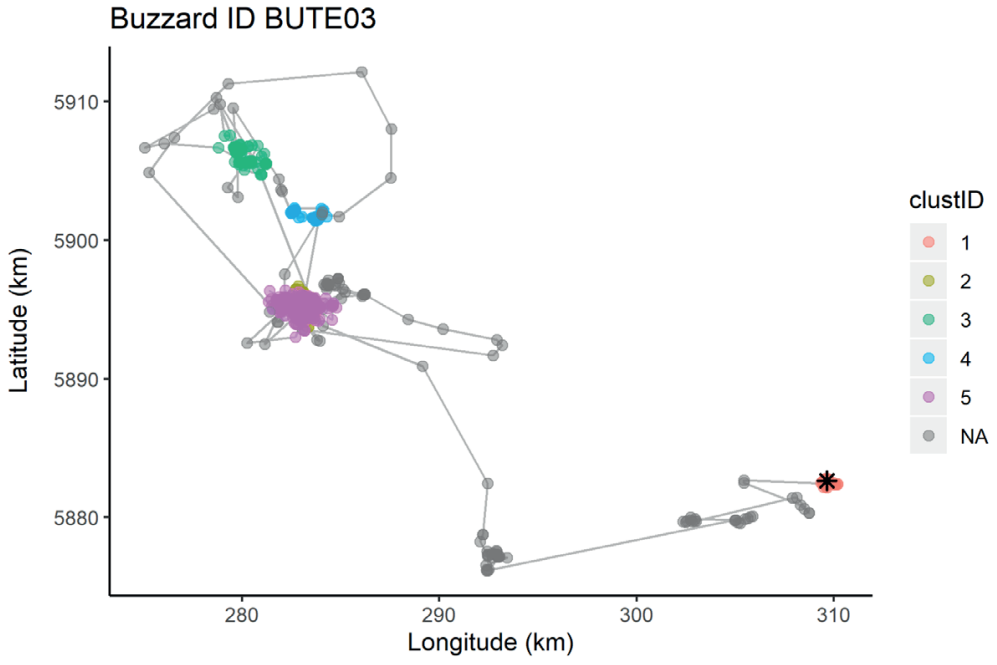
Buzzard ID BUTE03
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Longitude (km)
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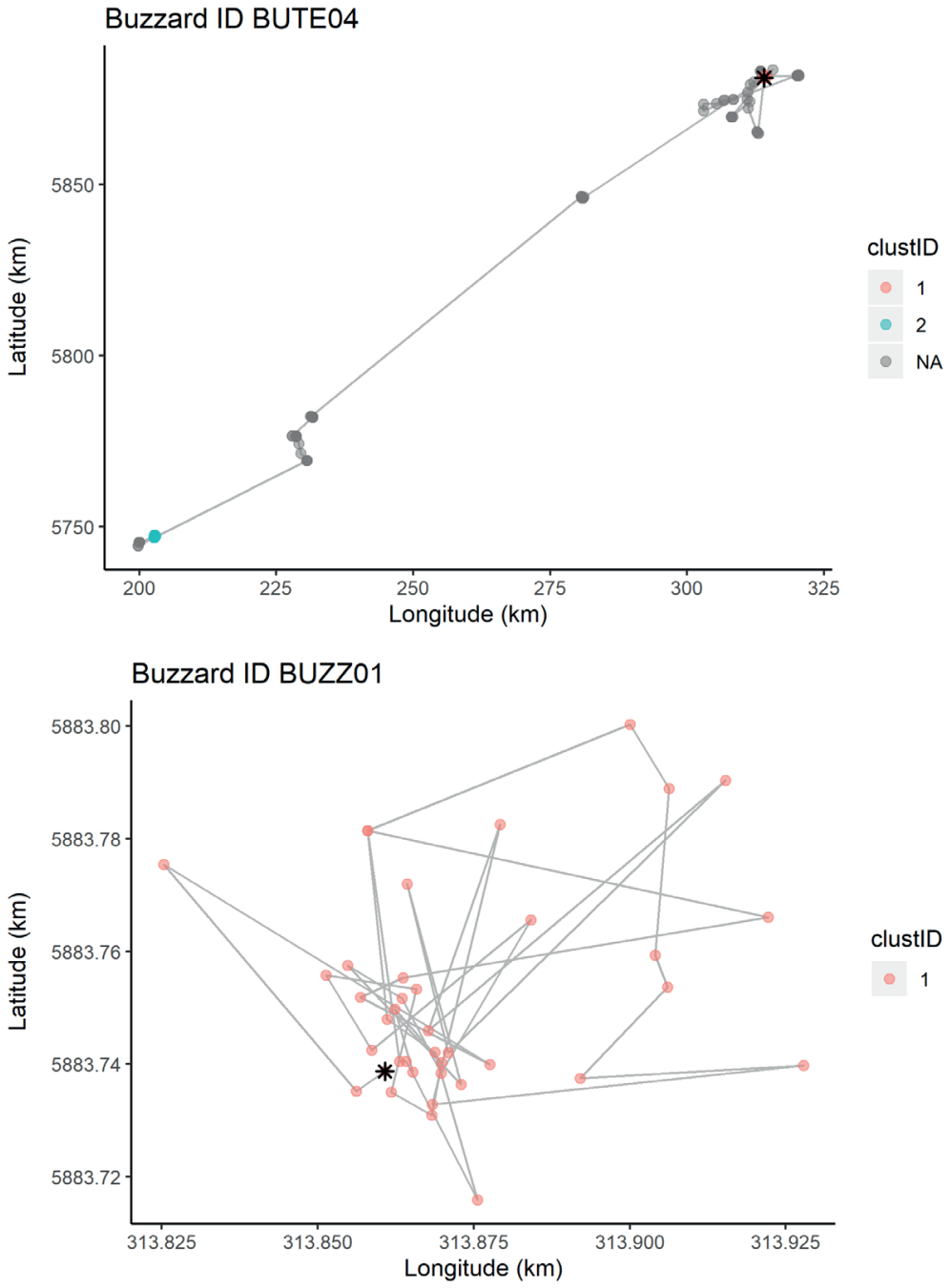

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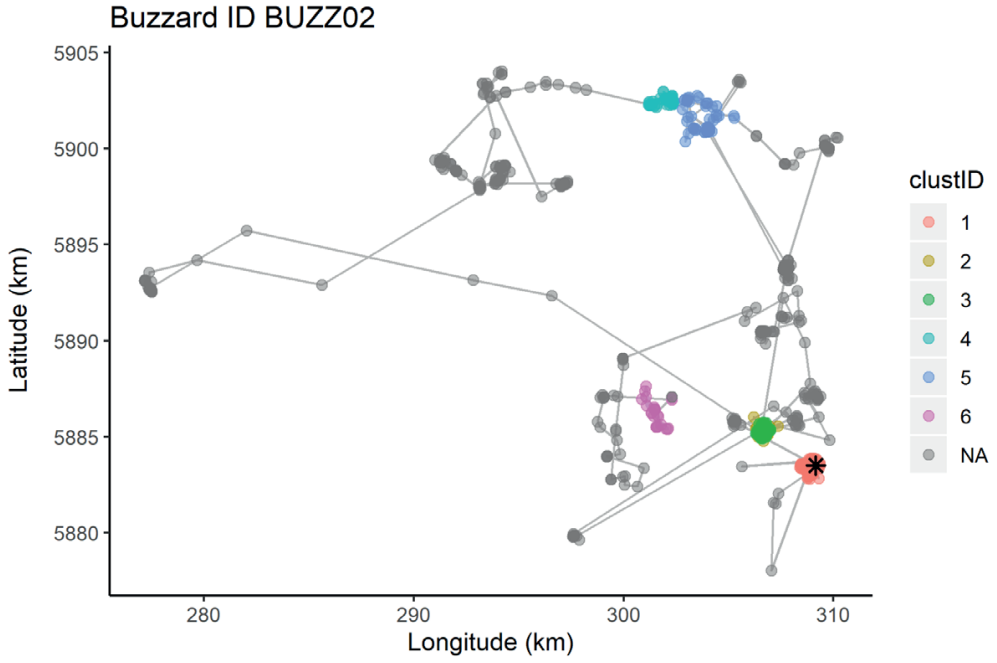
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Longitude (km)
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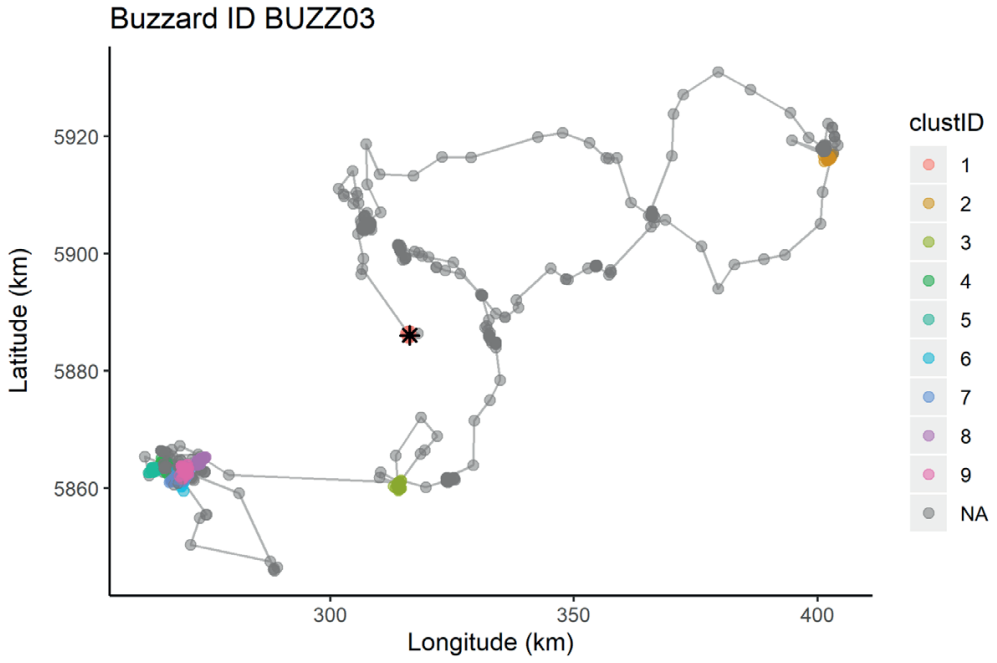
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Longitude (km)
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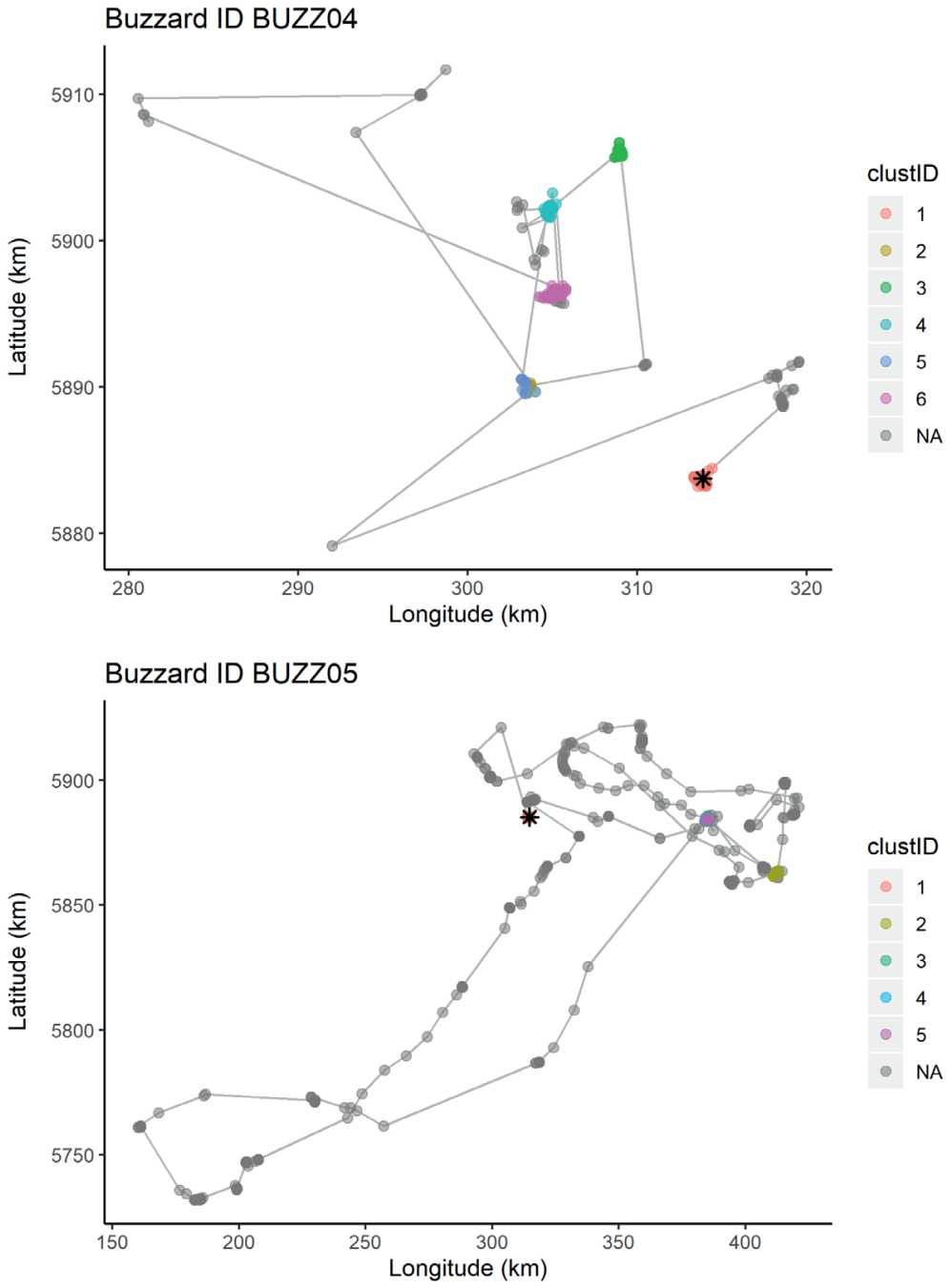

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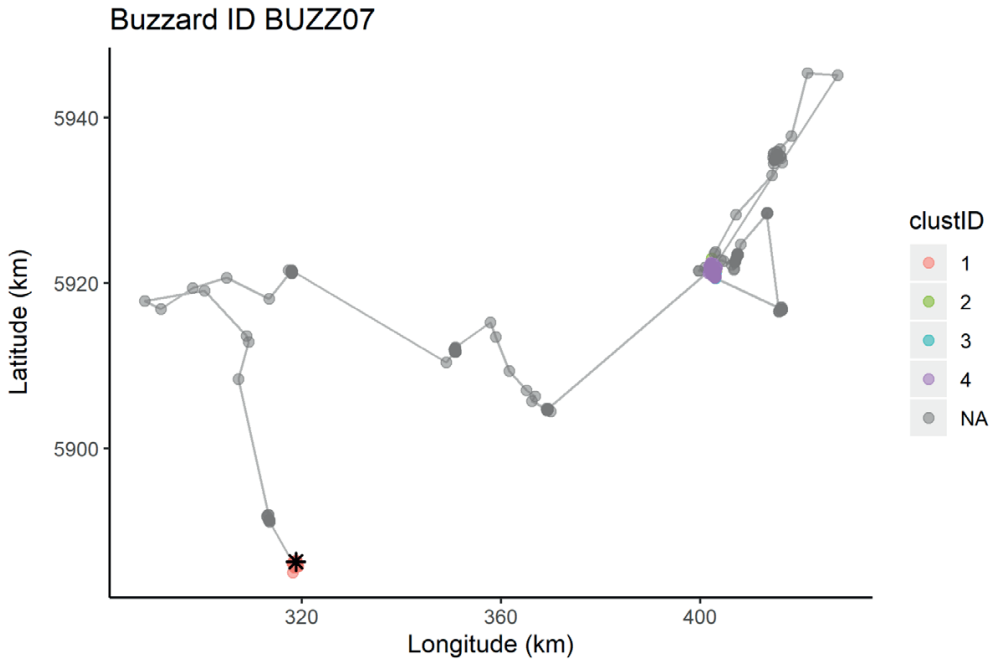
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Longitude (km)
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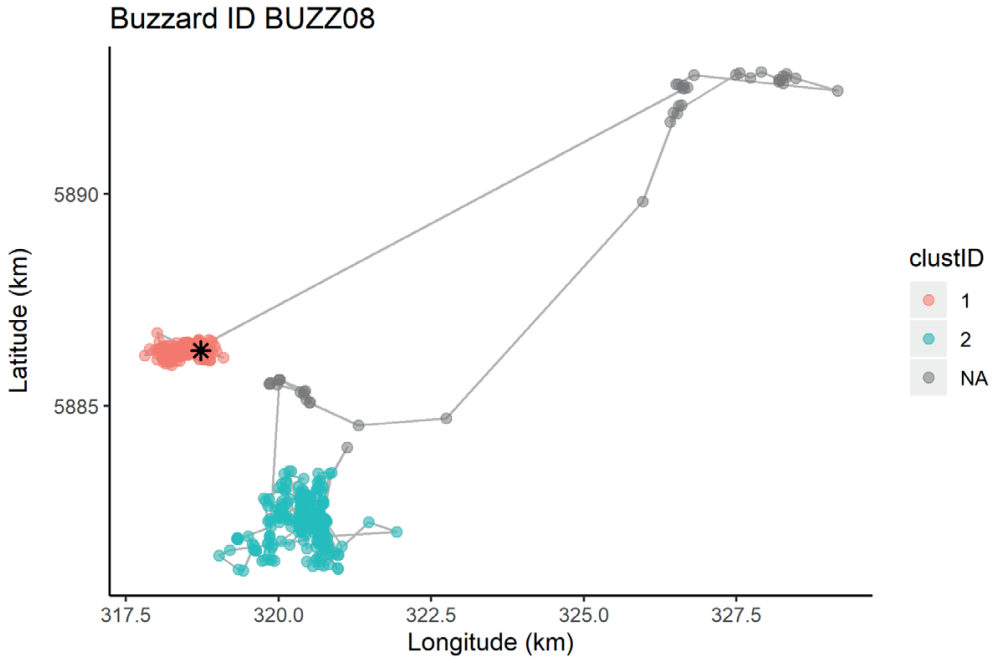
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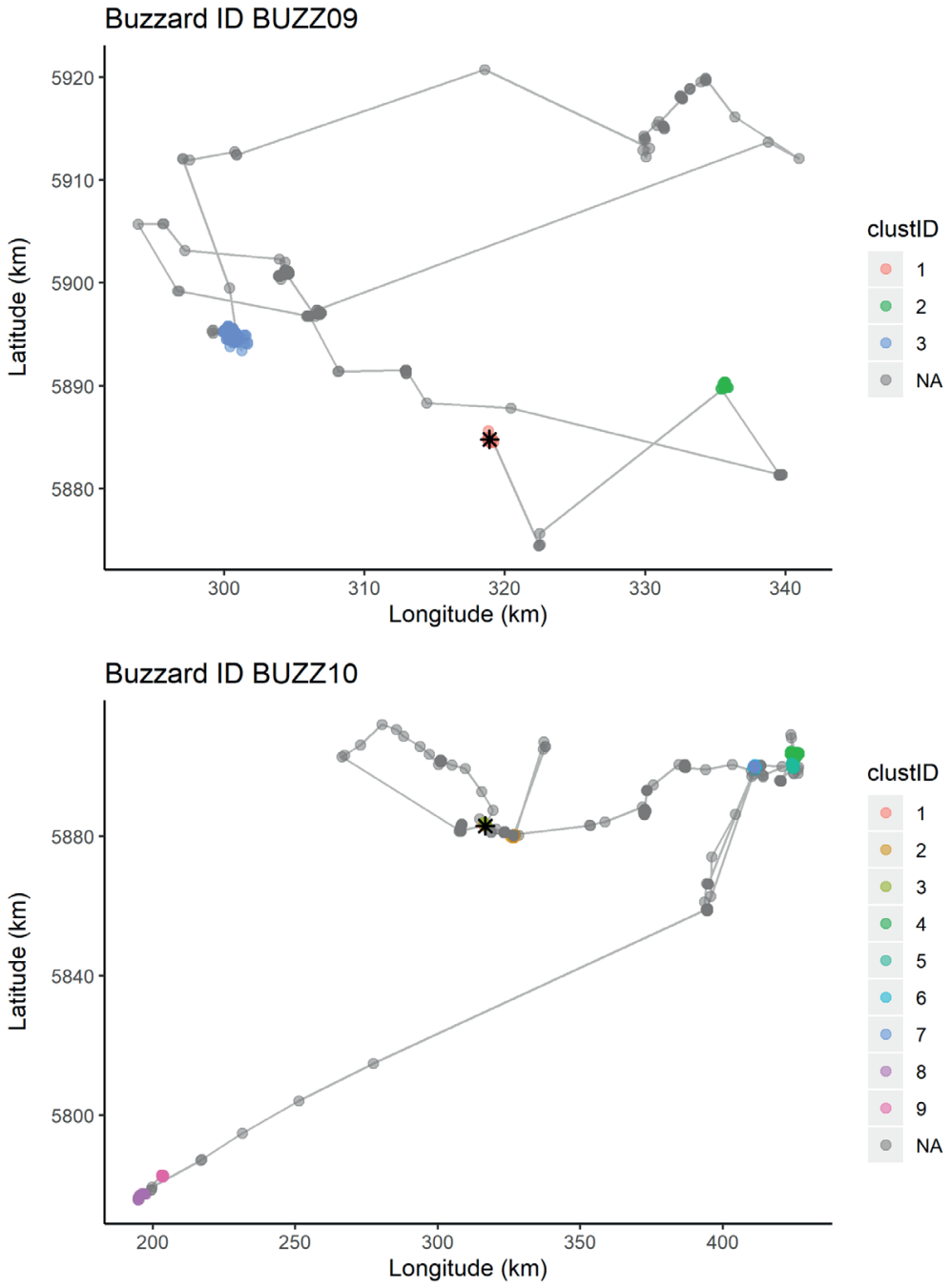

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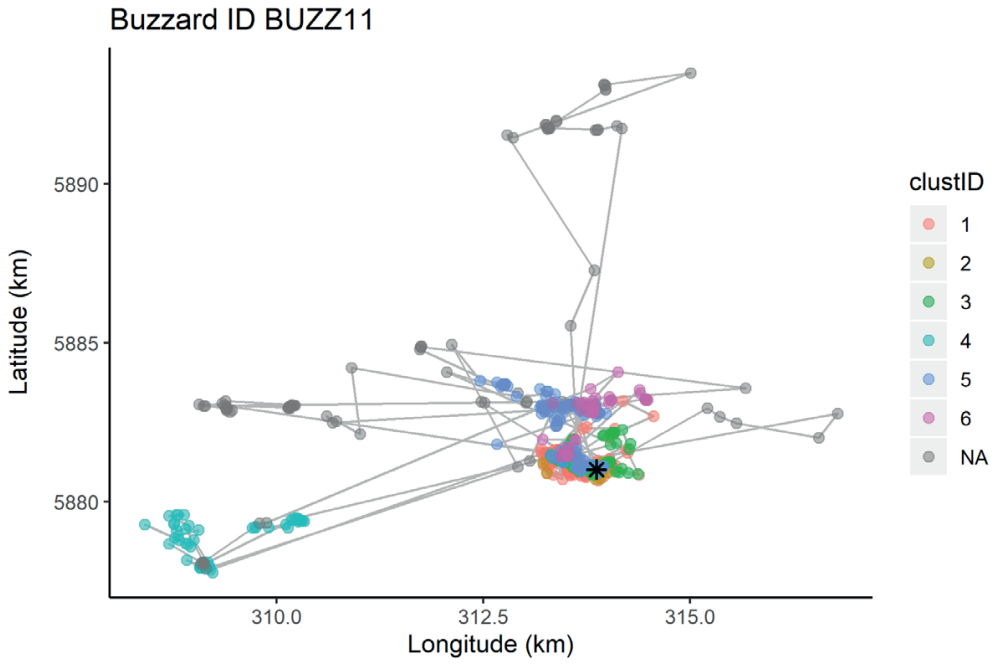
Buzzard ID BUZZ11
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Longitude (km)
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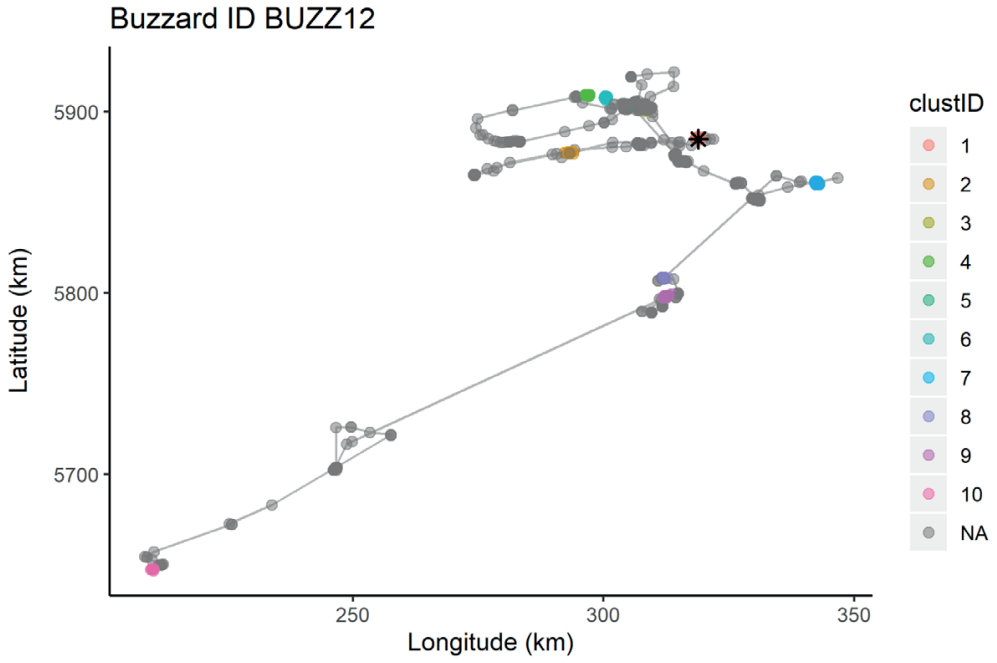
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Longitude (km)
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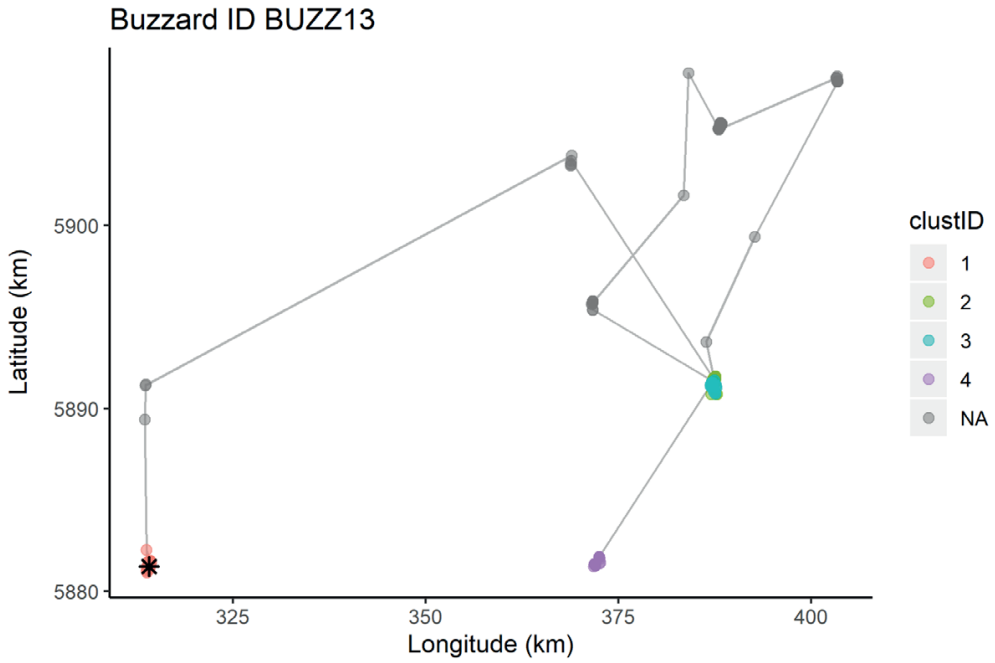

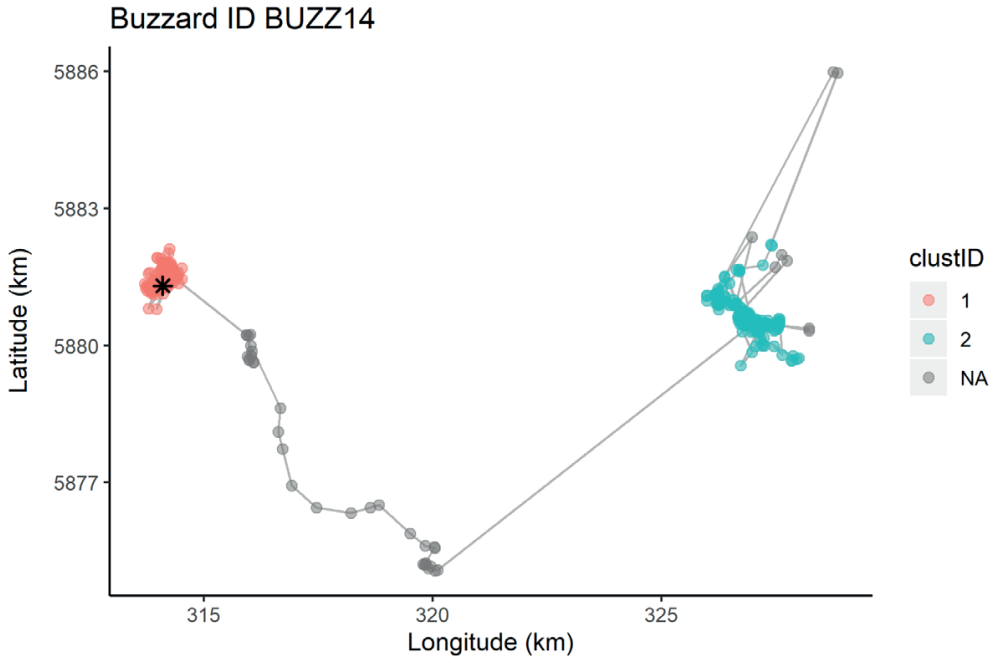

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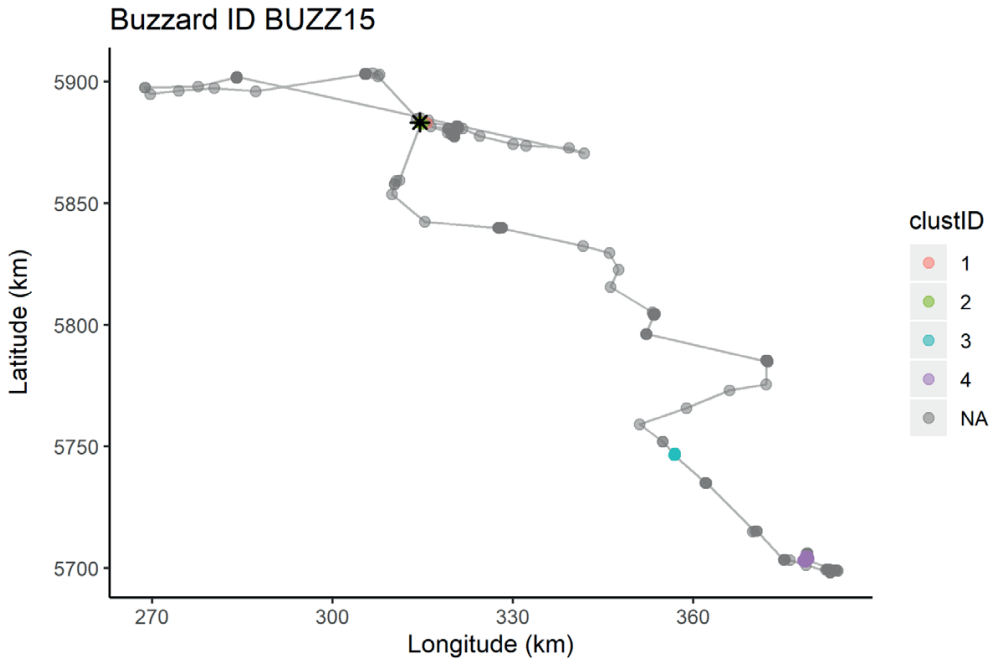
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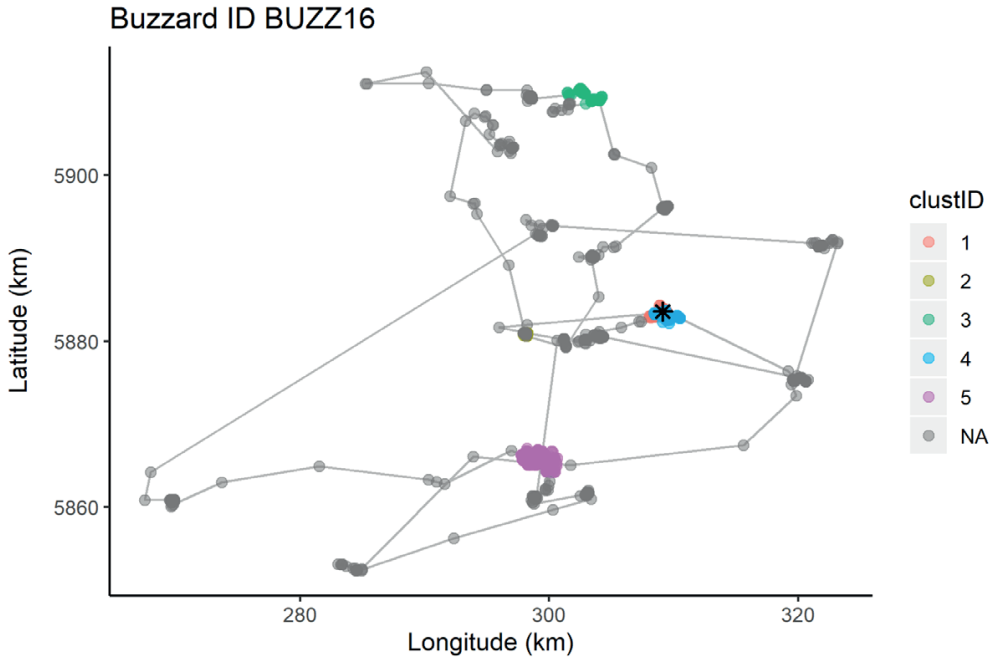
Buzzard ID BUZZ16
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Longitude (km)
clustID
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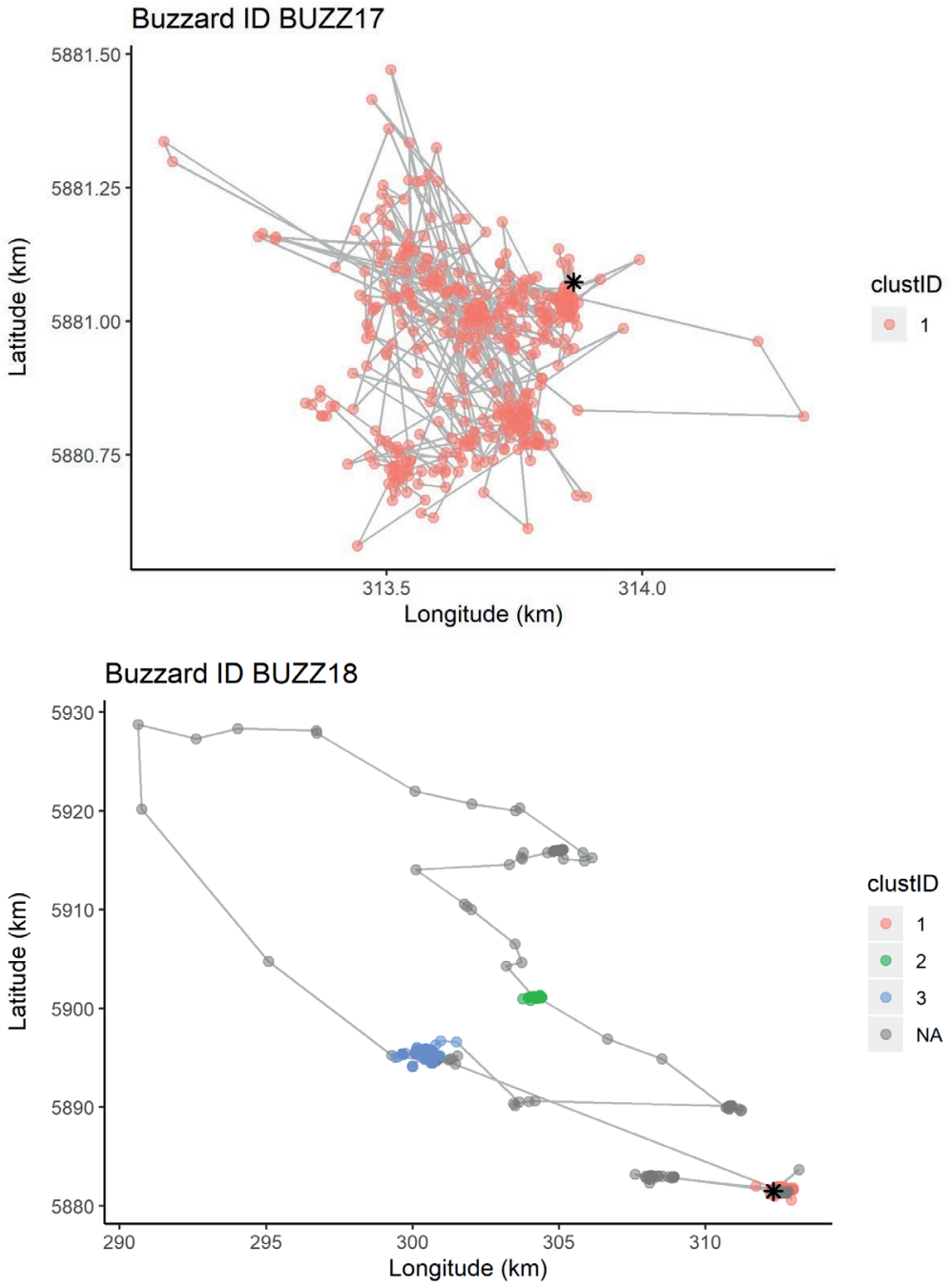

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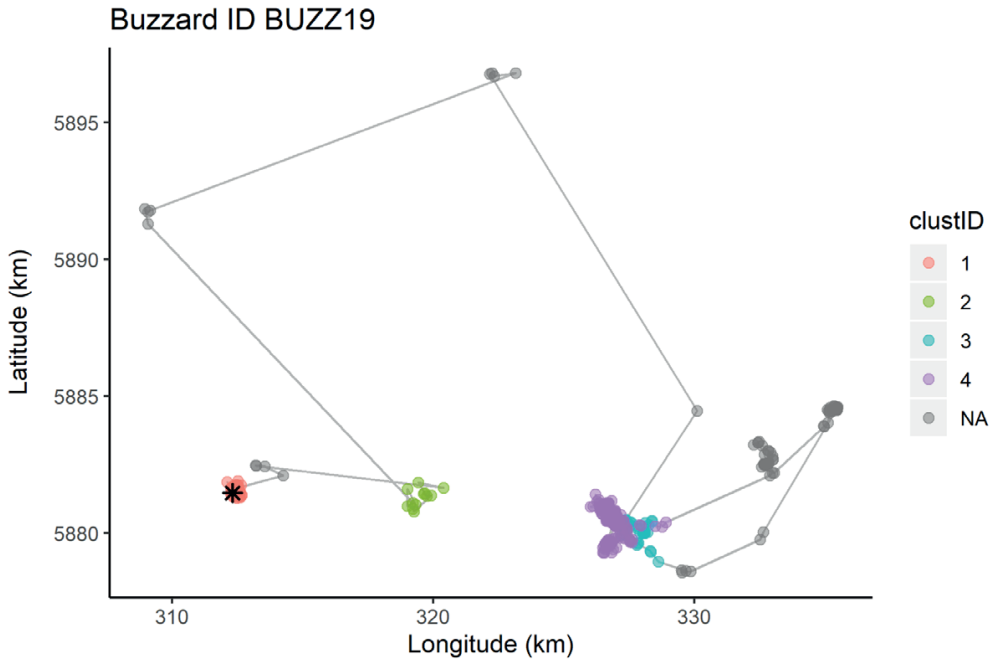
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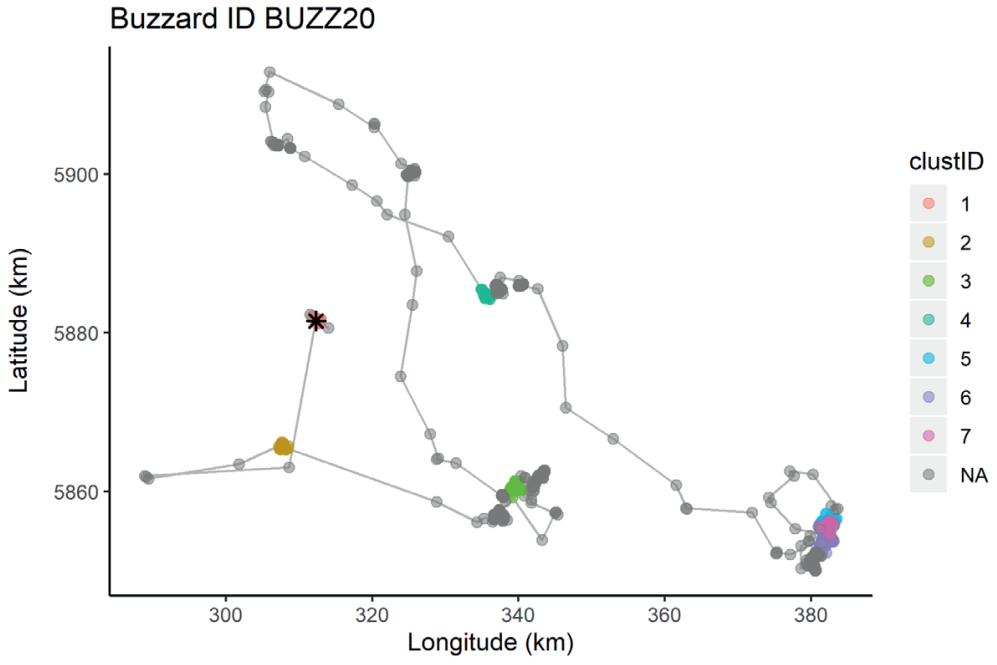
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380
Longitude (km)
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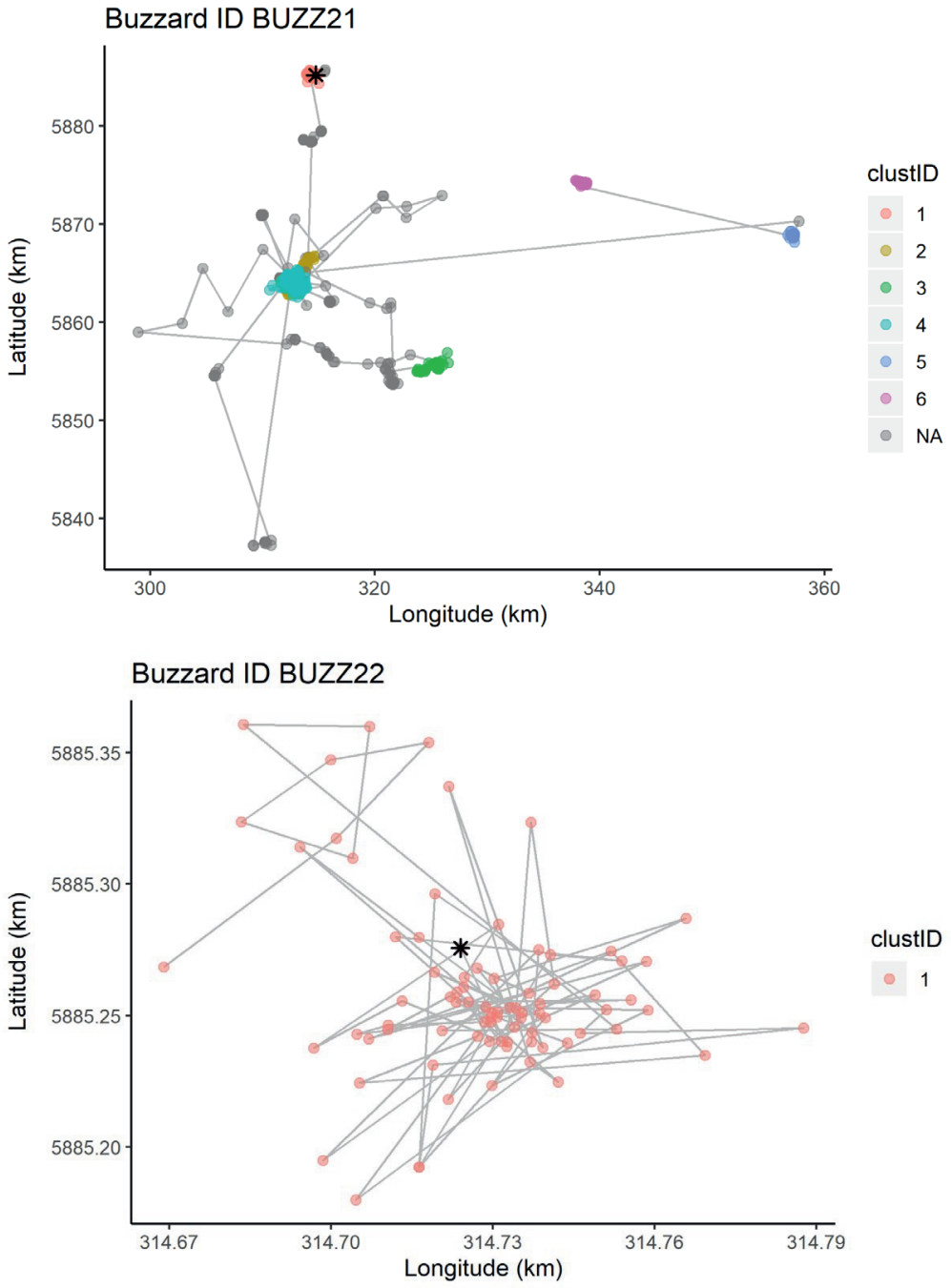
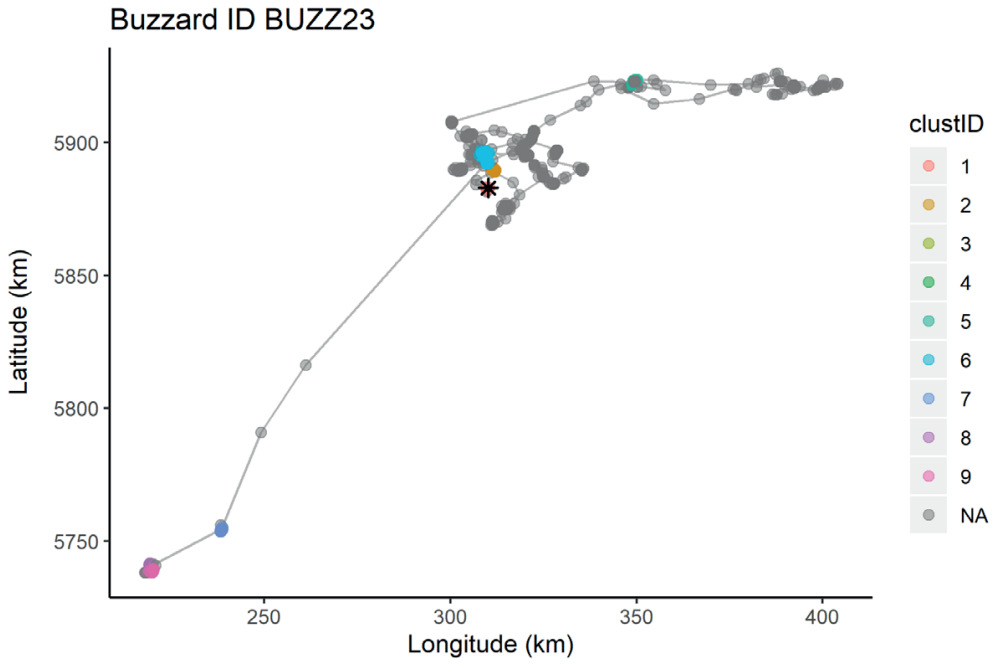


Figure S2: continued.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Longitude (km)
clustID
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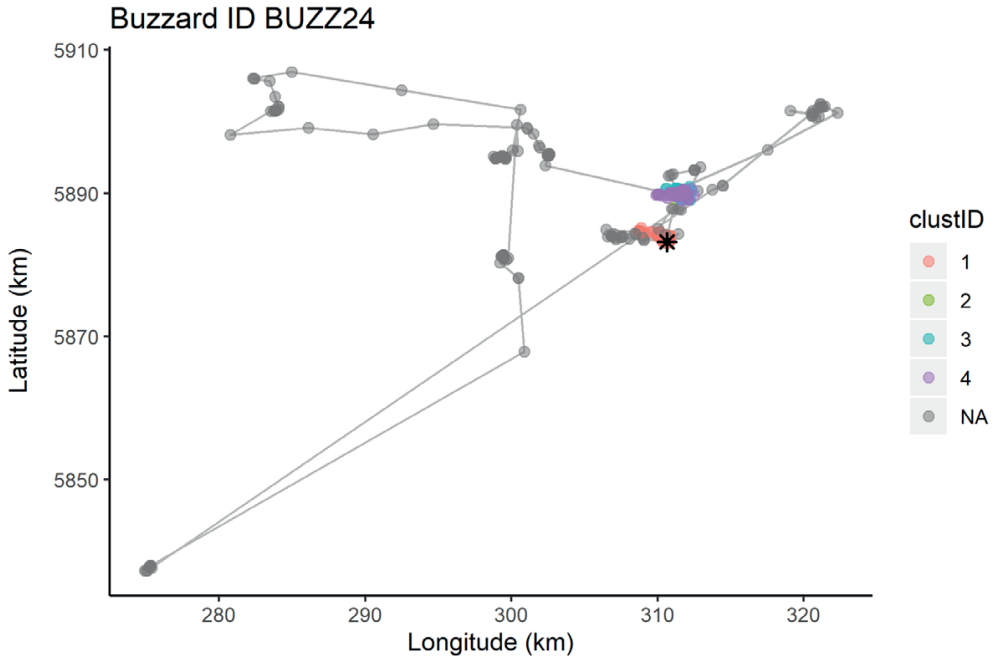
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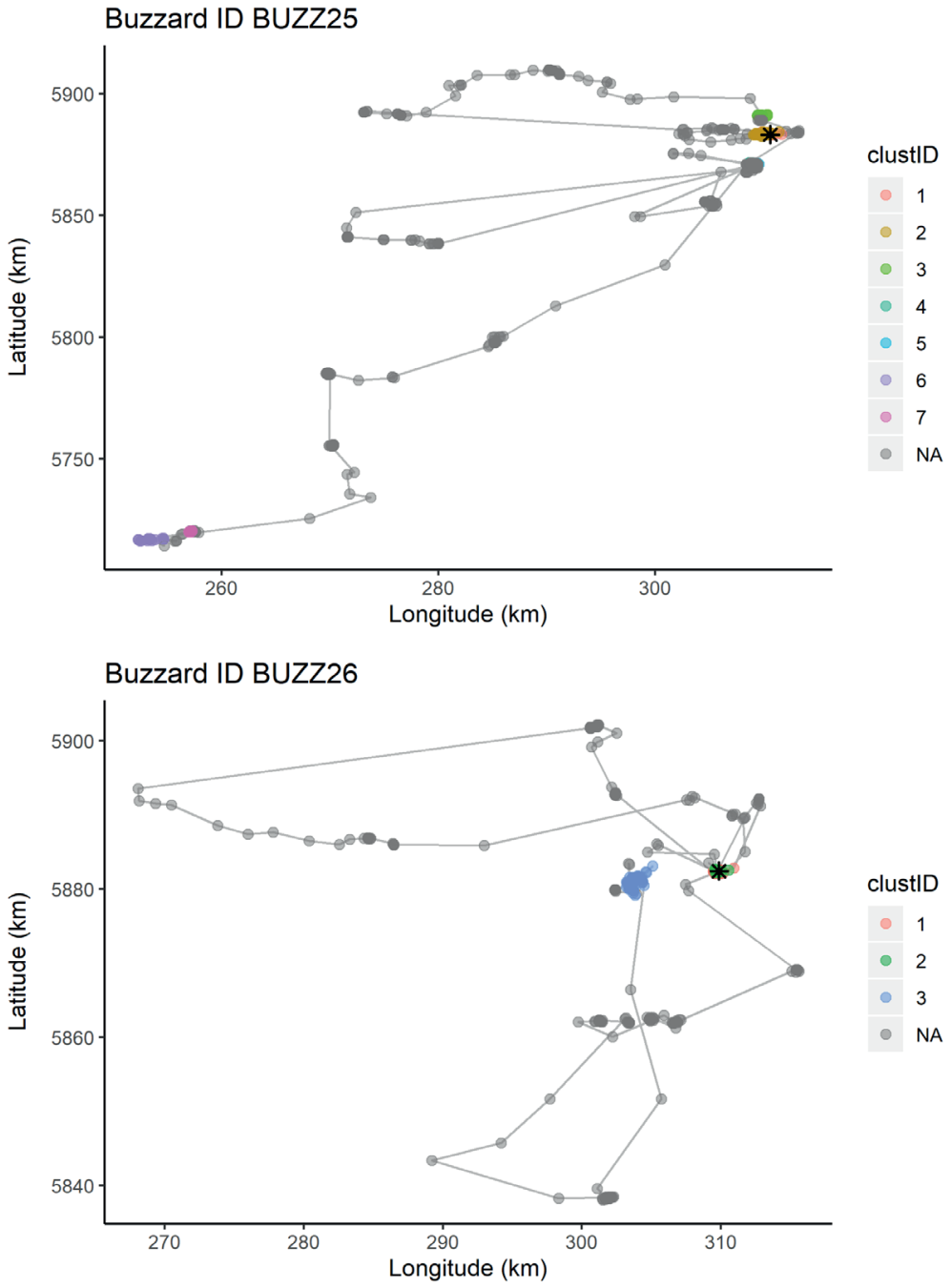
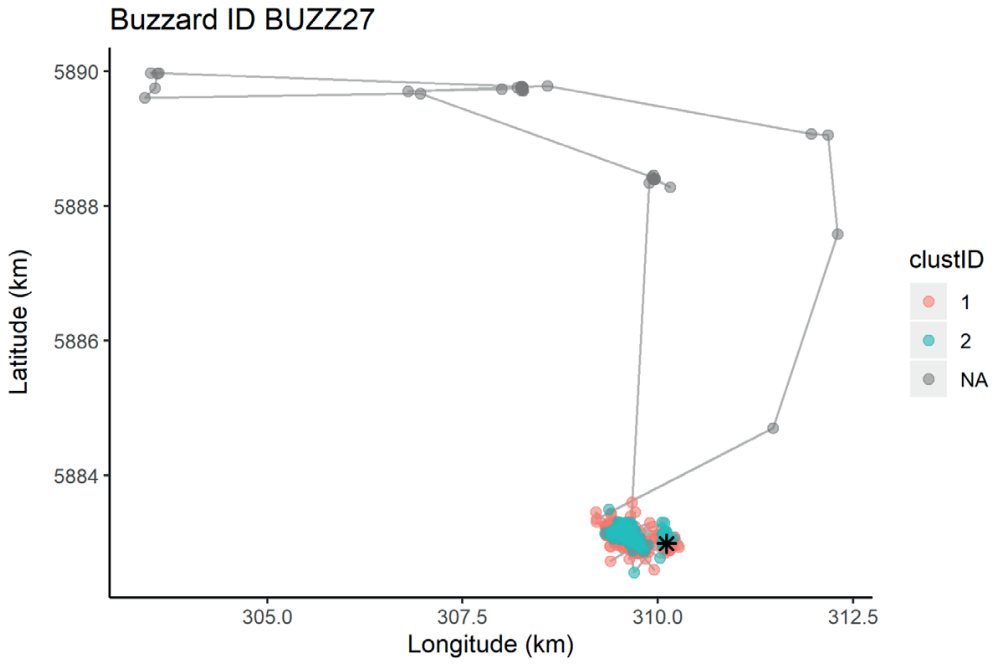


Figure S2: continued.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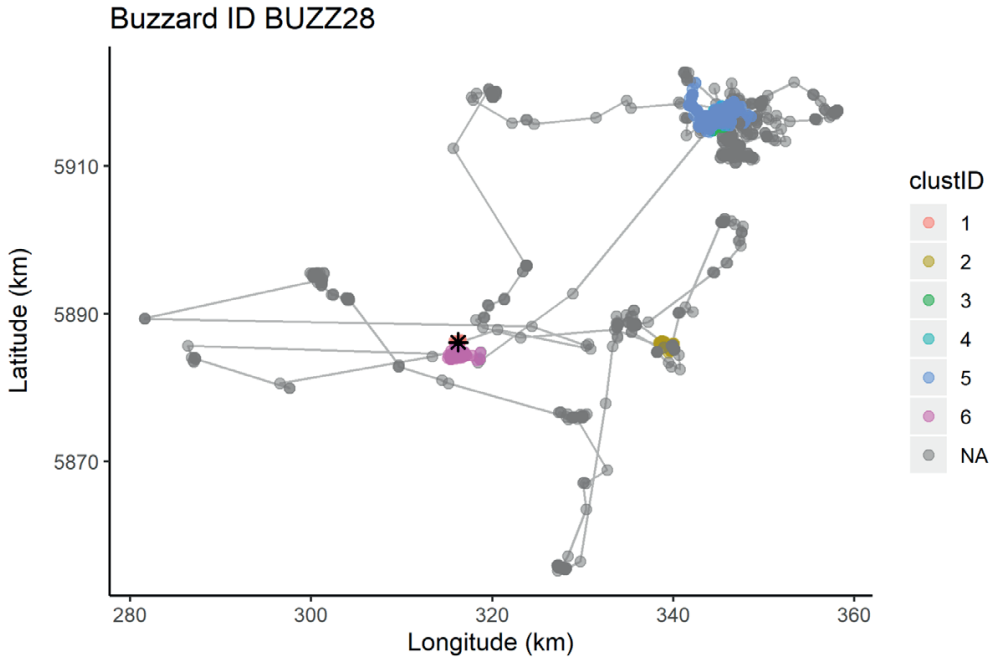
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Longitude (km)
clustID
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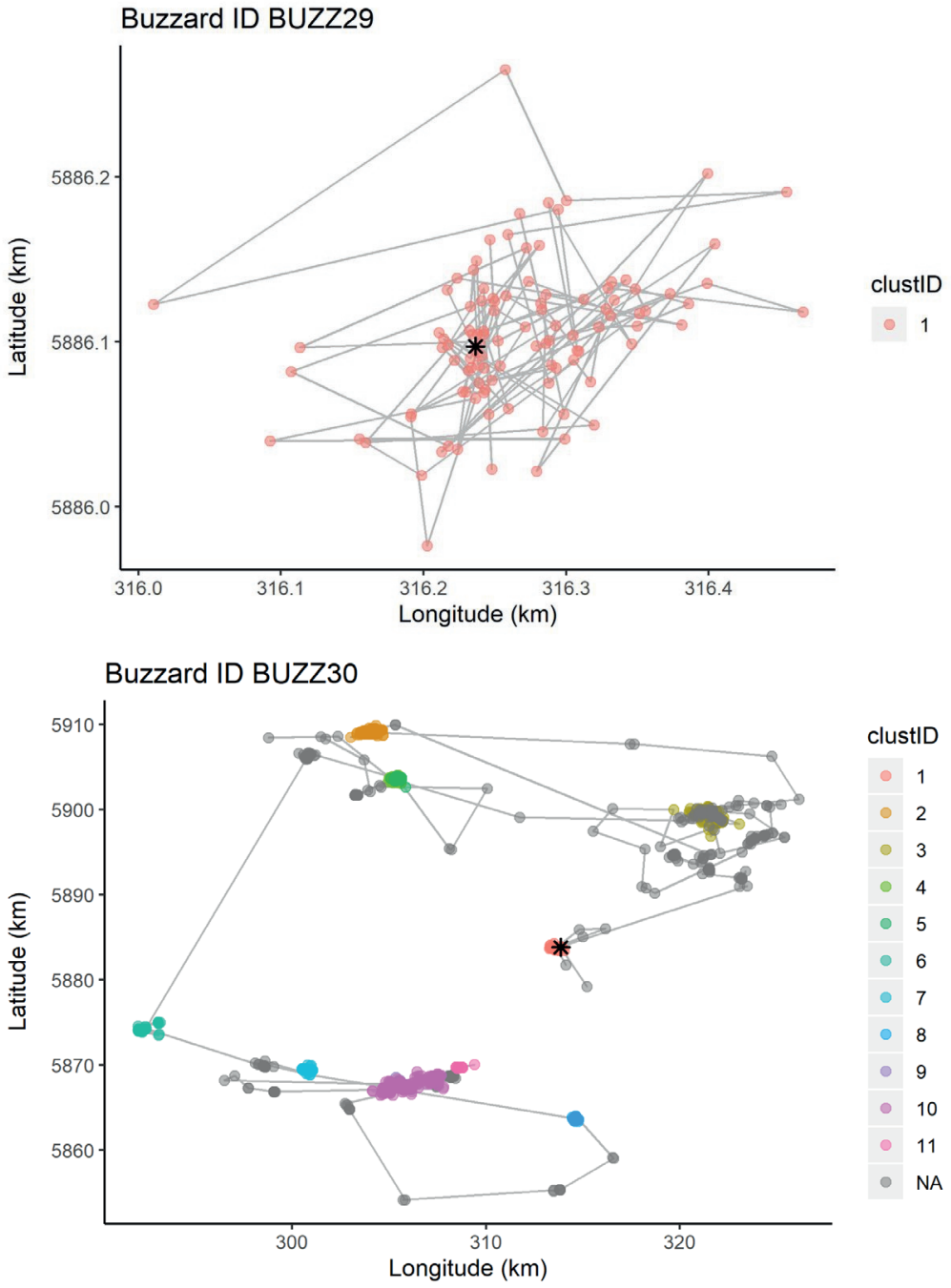

Figure S2: continued.

Table S1: Results of a linear mixed model explaining variation in emigration date (Julian) of 64 juvenile buzzards in relation to sex (female as reference category) and morph (continuous, dark to very light, scaled). We controlled for year (2015 as reference category), hatching date (Julian, scaled), brood size (numeric, range 1–3) and body condition index (scaled) and included nest and pair identity as random effects. Shown are the results from the simplified model, except for the interaction term estimate, which is from the full model.

Predictors	**Estimate**	***CI***	***P***
Intercept	238.08	223.64 – 252.51	
Sex: male	-5.30	-12.32 – 1.72	0.14
Morph*	-1.89	-5.81 – 2.02	0.34
Year: 2016	4.69	-2.57 – 11.96	0.20
Hatching date	5.89	1.73 – 10.04	**0.005**
Brood size	-6.90	-12.73 – -1.07	**0.020**
Body index	0.62	-2.87 – 4.10	0.73
Sex: male × Morph*	0.75	-6.81 – 8.31	0.85
Random Effects			
σ^2	180.73		
$\tau_{00\ nest}$	0.00		
$\tau_{00\ pair\ ID}$	4.61		
ICC_{nest}	0.00		
$ICC_{pair\ ID}$	0.02		
Observations	64		

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient
*on a three-morph scale

Table S2: Results of a linear mixed effect model explaining variation in number of residency areas visited of 64 juvenile buzzards during their first autumn (up to 200 days after tagging) in relation to sex (female as reference category) and morph (continuous, dark to very light, scaled). We controlled for year (2015 as reference category), number of recording days (scaled) and emigration date (Julian, scaled) and included nest and pair identity as random effects.

Predictors		**Estimate**	***CI***	***P***
Intercept		6.71	5.48 – 7.95	
Sex: male		0.53	-0.72 – 1.78	0.40
Morph*		-0.85	-1.52 – -0.19	**0.012**
Year: 2016		-1.47	-2.71 – -0.22	**0.021**
Recording days		0.89	0.24 – 1.54	**0.007**
Emigration date		-0.83	-1.47 – -0.20	**0.010**
Random Effects				
σ^2	4.58			
$\tau_{00\ nest}$	0.00			
$\tau_{00\ pair\ ID}$	1.55			
ICC_{nest}	0.00			
$ICC_{pair\ ID}$	0.25			
Observations	64			

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient
*on a three-morph scale

Table S3: Results of a linear mixed effect model explaining variation in tenure in 396 residency areas visited of 64 juvenile buzzards during their first autumn (up to 200 days after tagging) in relation to sex (female as reference category) and morph (continuous, dark to very light, scaled). We controlled for year (2015 as reference category), number of recording days (scaled) and emigration date (Julian, scaled) and included individual, nest and pair identity as random effects. Tenure in each of the residency areas was normalized by $\log_{10}$ transformation.

Predictors	**Estimate**	***CI***	***P***
Intercept	2.30	2.03 – 2.57	
Sex: male	-0.06	-0.32 – 0.21	0.67
Morph*	0.12	-0.02 – 0.26	0.11
Year: 2016	0.15	-0.12 – 0.43	0.28
Recording days	0.05	-0.08 – 0.19	0.44
Emigration date	0.13	-0.00 – 0.26	0.05
Random Effects			
σ^2	1.18		
$\tau_{00\ \text{tag ID}}$	0.00		
$\tau_{00\ \text{nest}}$	0.00		
$\tau_{00\ \text{pair ID}}$	0.07		
$\text{ICC}_{\text{tag ID}}$	0.00		
ICC_{nest}	0.00		
$\text{ICC}_{\text{pair ID}}$	0.06		
Observations	396		

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient
*on a three-morph scale

Table S4: Results of a linear mixed effect model explaining variation in distance from nest of the last residency area visited by 64 juvenile buzzards during their first autumn (up to 200 days after tagging) in relation to sex (female as reference category) and morph (continuous, dark to very light, scaled). We controlled for year (2015 as reference category), number of recording days (scaled) and emigration date (Julian, scaled) and included nest and pair identity as random effects. Distance from nest was normalized by square-root transformation. Shown are results from the simplified model, except for the interaction term estimate, which is from the full model.

Predictors		**Estimate**	***CI***	***P***
Intercept		7.31	5.28 – 9.34	
Sex: male		0.68	-1.52 – 2.88	0.54
Morph*		-0.15	-1.21 – 0.92	0.79
Year: 2016		-1.31	-3.56 – 0.93	0.25
Recording days		1.01	-0.13 – 2.15	0.08
Emigration date		-0.21	-1.34 – 0.91	0.71
Sex: male × Morph*		1.36	-0.81 – 3.53	0.22
Random Effects				
σ^2	18.06			
$\tau_{00\ nest}$	0.00			
$\tau_{00\ pair\ ID}$	0.00			
ICC_{nest}	0.00			
$ICC_{pair\ ID}$	0.00			
Observations	64			

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient
*on a three-morph scale

Table S5: We controlled for year (2015 as reference category), number of recording days (scaled) and emigration date (Julian, scaled) and included nest and pair identity as random effects. Cumulative distance travelled was normalized by square-root transformation. Shown are results from the simplified model, except for the interaction term estimate, which is from the full model.

Predictors		**Estimate**	***CI***	***P***
Intercept		9.69	7.17 – 12.22	
Sex: male		1.92	-0.81 – 4.65	0.17
Morph*		-0.55	-1.88 – 0.77	0.41
Year: 2016		-1.45	-4.24 – 1.33	0.30
Recording days		1.84	0.42 – 3.27	**0.011**
Emigration date		-1.06	-2.46 – 0.34	0.14
Sex: male × Morph*		1.29	-1.42 – 4.00	0.35
Random Effects				
σ^2	27.89			
$\tau_{00\ nest}$	0.00			
$\tau_{00\ pair\ ID}$	0.00			
ICC_{nest}	0.00			
$ICC_{pair\ ID}$	0.00			
Observations	64			

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient
*on a three-morph scale

Table S6: Results of a linear mixed model explaining variation in the proportion of forested habitat in the residency areas visited by 64 juvenile buzzards in relation to plumage colour morph (continuous, dark to very light, scaled). We controlled for sex (female as reference category) and year (2015 as reference category) and included nest and pair identity as random effects. The proportion of forested habitat was weighted by tenure and then normalized by arcsine-square-root transformation.

Predictors		**Estimate**	***CI***	***P***
Intercept		0.35	0.16 – 0.54	
Sex: male		-0.03	-0.10 – 0.04	0.38
Morph*		0.02	-0.06 – 0.10	0.63
Year: 2016		0.07	-0.02 – 0.15	0.12
Random Effects				
σ^2	0.01			
$\tau_{00\ nest}$	0.00			
$\tau_{00\ pair\ ID}$	0.03			
$ICC_{\ nest}$	0.07			
$ICC_{\ pair\ ID}$	0.60			
Observations	64			

σ^2 = Variance
τ_{00} = Ratio of population variance between groups
ICC = Intraclass Correlation Coefficient
*on a three-morph scale

Table S7: Number of days with at least one position sent of all 72 juvenile buzzards tagged in our study. Additional information is sex, morph scored on a seven-scale, year of tagging and identity of the parents.

Tag ID	Sex	Morph	Year	Pair ID	N days
550	male	4	2015	632.633	66
550b	male	2	2016	624.402	23
551	female	6	2015	630.303	64
552	male	5	2015	632.633	79
553	female	6	2015	630.303	95
553b	male	4	2016	700.345	33
554	female	4	2015	040.541	96
555	female	4	2015	560.561	72
556	male	6	2015	630.303	10
557	male	6	2015	S573	52
558	male	2	2015	040.541	110
559	female	2	2015	045.316	108
560	female	6	2015	461.575	78
561	female	4	2015	104.464	7
562	male	2	2015	542.648	109
562b	male	6	2016	126.127	105
563	male	3	2015	442.443	104
564	male	3	2015	S570	8
565	male	5	2015	483.484	126
566	female	4	2015	573.345	16
567	male	4	2015	478.479	122
567b	female	7	2016	525.526	64
568	male	2	2015	045.316	109
568b	female	7	2016	525.526	3
569	male	4	2015	560.561	111
569b	male	2	2016	542.648	116
570	male	4	2015	401.012	105
571	female	3	2015	478.479	68
572	male	4	2015	573.345	77

Tag ID	Sex	Morph	Year	Pair ID	N days
573	male	5	2015	S573	90
574	male	2	2015	461.575	91
574b	male	2	2016	542.648	75
575	male	7	2015	401.012	74
576	female	3	2015	542.648	45
576b	female	7	2016	525.526	44
577	male	3	2015	461.575	73
578	female	2	2015	104.464	95
578b	male	4	2016	624.402	114
579	male	4	2015	573.345	59
BUTE01	male	6	2015	536.587	115
BUTE02	female	7	2015	525.526	116
BUTE03	male	6	2015	650.651	124
BUTE04	female	5	2015	536.587	86
BUZZ01	female	7	2016	306.307	7
BUZZ02	female	3	2016	370.678	118
BUZZ03	male	3	2016	040.541	136
BUZZ04	female	6	2016	306.307	140
BUZZ05	female	6	2016	514.549	148
BUZZ07	female	6	2016	500.501	105
BUZZ08	male	6	2016	500.501	108
BUZZ09	male	4	2016	632.633	95
BUZZ10	female	2	2016	045.316	136
BUZZ11	male	3	2016	385.646	93
BUZZ12	male	4	2016	632.633	129
BUZZ13	male	3	2016	536.587	59
BUZZ14	male	4	2016	536.587	107
BUZZ15	female	2	2016	104.464	104
BUZZ16	male	5	2016	370.678	139
BUZZ17	male	3	2016	385.646	61
BUZZ18	female	6	2016	483.484	122
BUZZ19	male	3	2016	483.484	89

Tag ID	Sex	Morph	Year	Pair ID	N days
BUZZ20	male	6	2016	483.484	94
BUZZ21	male	5	2016	514.549	118
BUZZ22	male	7	2016	514.549	23
BUZZ23	male	4	2016	672.059	173
BUZZ24	female	2	2016	461.575	102
BUZZ25	female	5	2016	461.575	135
BUZZ26	male	6	2016	650.651	101
BUZZ27	female	3	2016	672.059	121
BUZZ28	male	4	2016	040.541	162
BUZZ29	male	4	2016	040.541	27
BUZZ30	male	5	2016	306.307	139

6

General discussion

Elena Frederika Kappers

Ecology and evolution are closely interconnected, since evolution concerns changes in genetic diversity of populations over time and ecology concerns changes in the distribution and interactions of populations over time. In fact, evolutionary processes take place in an ecological context because of the relationships between organisms and the environment. My thesis is in this field of evolutionary ecology, on a highly studied but still poorly understood phenomenon, the maintenance of intraspecific variation in colour polymorphism.

Persistent colour polymorphism, i.e. differences in coloration in the same age and sex class within a population, has historically been used to understand the mechanisms that help to generate within- and between-species diversity. It has been the subject of many studies investigating the maintenance of genetic diversity (e.g. in birds, Roulin 2004b). Among bird species, raptors of the genus Buteo show a disproportionately high frequency of colour polymorphisms (60% of species are polymorphic) (Galeotti et al. 2003). These polymorphisms are interesting from an evolutionary perspective, because they are heritable and hence a good model for understanding mechanisms preserving genetic variation. A proposed selective process that contributes to the maintenance of colour polymorphism is balancing selection. There are two mechanisms by which balancing selection works to maintain this polymorphism. These are heterozygote advantage and frequency-dependent selection. These processes, coupled with environmental heterogeneity appear to be important in promoting colour polymorphism.

The Common buzzard *Buteo buteo* is an interesting model for understanding how genetic variation is maintained in a polymorphic species. This bird of prey is a common species, it has a large geographic range, it lives in spatially and temporally heterogeneous environments and it occupies diverse habitats. In Common buzzards, variation in plumage colour has been reported to be maintained by a heterozygote advantage in a German population: heterozygote intermediates had higher fitness than homozygote light and dark morphs. Interestingly, and unexpectedly, these German buzzards were mating in a maladapted fashion: they preferred partners of similar plumage, whereas from a fitness perspective, light individuals should pair up with dark individuals and thereby producing the most fit intermediate offspring. This observation was part of the reason why I started my study, as these results required replication to see whether these patterns are of more general nature.

The goal of this thesis was to examine the variation in and the maintenance of colour polymorphism in a Dutch population of Common buzzards. First, I described colour variation to understand if and how this phenotypic trait is inherited. Then I looked at fitness differences among morphs and investigated temporal and spatial variation of colour polymorphism in this species.

In this final chapter I will summarize the main results of the previous chapters and put them in broader context with the previous literature, followed up by a discussion on what would be required to still better understand the maintenance of colour polymorphism in this species. To do so, I will invoke the help of yet unpublished preliminary data which show the importance of large-scale spatial aspects.

Results reported in previous chapters

In **chapter 2** I examined whether discrete morphs exist or whether plumage colour variation is more continuous in Common buzzards. Using image analysis, I showed that variation is continuous and unimodal, ranging from very dark to very light individuals. Because variation appears to be continuous, scoring systems containing more categories than the three in previous studies would better capture the underlying variation. As previous studies have used different scoring systems with fewer morphs, I showed how a seven-morph scale relates to the previously described three-morph scale. I suggested that the seven-morph scale describes the continuous colour variation reasonably well. I used photographs of the same individuals taken at different ages, including both males and females, and showed that the observed variation is highly repeatable within individuals, even though plumage gets somewhat darker from juvenile to adult age. I detected no sexual difference in plumage colour. The second chapter contributes to the increase in basic knowledge on plumage colour variation in the species.

Using an animal model approach for quantitative genetics, in **chapter 3** I showed that variation in plumage colour is 82% heritable, in about two hundred families of a Dutch population when comparing fledglings with their parents. However, I found no support for a simple Mendelian one-locus two-allele model of inheritance, as suggested by Krüger et al. (2001). In fact, the proportion of observed offspring morphs significantly differed from the expectations from such an inheritance mode, showing in general a higher proportion of intermediate offspring for assortatively mated pairs, and higher proportions of extreme offspring in disassortatively mated pairs. The results of the third chapter suggest that melanic plumage colour in Common buzzards should be considered a quantitative polygenic trait. Interestingly, the data provided by Krüger et al (2001) did not deviate from the patterns observed in our population, and the reason we came to a different conclusion is partly due to larger sample sizes.

In **chapter 4**, I took advantage of 20 years of life-history data collected by Christiaan de Vries and Anneke Alberda to replicate earlier studies on fitness consequences of colour polymorphism in this species (Krüger et al. 2001). I first examined morph differences in adult apparent survival by using sight-resight data in the program MARK. I found only weak support for morph-dependent survival rates for both males and females, with intermediate adults having slightly higher survival. Secondly, I looked at mate choice and I observed positive assortative mating for colour morph. Moreover, I found that assortative pairs were more likely to produce offspring than disassortative pairs, and their pair bonds lasted longer. Then, I looked at different fitness components of the morphs, specifically at breeding success, annual number of fledglings produced and cumulative reproductive success. I found that cumulative reproductive success differed among morphs, with the intermediate morph having highest fitness. Lastly, in our long-term population study I observed a phenotypic change with an increasing proportion of intermediate morphs over time.

After these detailed studies on the ecology of breeding adults, **chapter 5** gives an overview on the first months of life of juvenile buzzards dispersing from their natal sites. This period of the life has been hardly studied, and may actually be important in fitness studies.

I studied the effects of plumage coloration on natal dispersal behaviour in individuals leaving our study population in The Netherlands. More specifically, I looked at emigration timing and exploratory behaviour in the first months of wandering. For the same period, I also investigated the effect of plumage coloration on habitat choice. To do this, I used GPS-transmitter data collected from juveniles leaving the natal nest and tested whether plumage coloration influenced number of areas visited, tenure in areas, cumulative distance among areas, distance of settlement from nest in first winter and proportion of forested habitat chosen. I found that coloration was associated with the number of areas visited, but not with other traits. Darker individuals visited a higher number of areas during the first months of dispersal compared to lighter individuals, likely suggesting a behavioural difference among morphs. However, the idea of matching phenotype with habitat choice (i.e. darker individuals using more forested habitats, whereas light individuals more open habitats) was not supported.

Conclusions

As my thesis was initially inspired by earlier studies on fitness consequences of colour polymorphism in Common buzzards (Krüger et al. 2001; Boerner and Krüger 2009; Jonker et al. 2014), I will contextualize my results and discuss differences and commonalities between studies.

Continuous variation and inheritance

Research addressing evolutionary questions about the maintenance of plumage colour variation requires estimating both the inheritance of, and selection on the trait. However, at first a good description of the variation is needed. For many polymorphic species in which a continuous variation of the coloration has been recognized, a classification system with few morph categories has often been used for studies of evolutionary ecology. Reducing continuous variation to a few categories can be extremely convenient and useful in field studies and to allow comparisons of research on the same species. The variation of a heritable phenotypic trait described by a continuous curve is often indicative of an underlying polygenic system of genetic control, where many genes with minor and additive effects are involved (Mather 1949). Instead, in case of really distinct, discrete colour morphs, one or a few genes are often involved that code for the variation (Mundy 2005). In this case, the use of phenotypic categories for the study of genotypes is a good proxy and relatively simple models can be used to assess selection on the trait. However, if variation is continuous but reduced to too few phenotypic categories, a misinterpretation of the results can be incurred if referring to the genotypes and the evolution of the genetic variation. Deductions based on the genetic inheritance system and mechanisms of natural selection may be too speculative.

In previous work on Common buzzards, continuous colour variation was simplified to few (=three) morphs and selection favouring intermediately coloured individuals was shown,

suggesting that these were the heterozygotes in a one-gene, two-allele system (Krüger et al. 2001).

I chose to use the three-morph classification scheme to be able to compare the results of previous studies with mine, even after not finding distinctive multimodality in buzzard plumage colour variation. Also, I used the seven-morph classification scheme to look at phenotypic variation with a scale closer to the continuous one. It is still possible that the selection dynamics could be understood when simplifying colour variation to few morphs, for example when there is one gene with a major effect and many with minor effects, resulting in continuous variation. But this would require that the used classifications align well with the underlying variation in the major gene. I made use of the social pedigree of the Dutch population to look at the heritability of the trait and I found that in our buzzard population plumage colour was highly heritable, independent of sex, and not influenced by environmental factors. This implies that selection can act on the trait and that the variance is either selectively neutral or a mechanism exists that keeps the polymorphism stable. When looking at the inheritance of plumage coloration (scored in different scenarios), I found that, in any scenario, the trait does not follow a one-locus two-alleles system of the simple Mendelian inheritance pattern. The genetic basis of melanic coloration in Common buzzards is likely composed by more genes. However, without carrying out molecular genetic analyses, one can only affirm that a certain genetic basis is unlikely and exclude the simple Mendelian inheritance system. Without molecular genetic analyses, it remains difficult to define which morph is heterozygote and which homozygote, regardless of whether they belong to a discrete coloration scale with three morphs, seven morphs or to a continuous gradient of coloration.

Fitness consequences

Although the genetic basis of the (continuous) colour variation is still unresolved, I used the three-morph classification scheme to test whether there were ecological differences among morphs and if I could replicate the heterozygote advantage as shown in a German population of buzzards (Krüger et al. 2001). Under this hypothesis, I expected to find higher survival and reproduction rates for the intermediate morph. I found that the three morphs differed only weakly in apparent survival, as had been similarly found by Jonker and colleagues (2014), and in both cases the intermediate morph was the one slightly advantaged (table 6.1).

The overall annual survival was higher in the Dutch population than in the German population, but it is not clear whether these differences are the result of ecological differences (e.g. predation rates, persecution), or are due to methodology (mostly sightings in Germany, whereas based on moulted feathers in The Netherlands) among study populations (table 6.2).

		German studies					This thesis			
		Morph			*N*	Reference	*Morph*			*N*
		Light	*Interm.*	*Dark*			*Light*	*Interm.*	*Dark*	
Adult frequency	♀	28.9%	65.1%	6%	106?	Tab.1, Krüger et al 2001	23.7%	48.5%	27.8%	266
	♂	32.4%	57.9%	9.7%	132?		16%	50.8%	33.2%	244
Adult frequency		37.5%	48.3%	14.2%	555	Mueller et al 2016				
Adult survival	♀	71%	76%	67%	669	Fig.1, Jonker et al 2014	87%	89%	88%	266
	♂	77%	78%	72%	670		86%	91%	89%	244
LRS / CRS	♀	1.8 (n=82)	4.5 (n=129)	1.3 (n=29)	240	Fig.1, Boerner et al 2009	3.2	3.5	2.8	266
	♂	1.8 (n=84)	3.7 (n=153)	1.7 (n=37)	274		3.7	4.4	3.9	244

Table 6.1: Comparison between the morph-dependent fitness results of the previous studies on a German population and the results from this thesis.

		German studies			This thesis	
		Rate	*N*	Reference	Rate	*N*
Annual resighting	♀	?	669	Jonker et al 2014	87%	266
	♂	?	670		86%	244
Adult survival	♀	74%	?	Fig. 4a (subset from period 1999-2010), Jonker et al 2014	88%	266
	♂	80%	?		90%	244
		Pearson's correlation coefficient			Pearson's correlation coefficient	
Mate choice		0.27	391 unique pairs	Calculated form data in Table 1b, Krüger et al 2001	0.13	400 unique pairs

Table 6.2: Comparison between the results of the previous studies on a German population and the results from this thesis. Note that the degree of assortative mate choice seems to be lower in the Dutch population, but that it is not completely clear whether the methodology is the same. If we consider all breeding pairs (i.e. also including repeated observations of the same pair), our correlation coefficient is 0.24. The question marks are for values not reported in the original study.

The methodology used to estimate survival was the same for both studies, but the probability of adult re-sighting was not reported for the German population. The different ecological context, as the presence of a top predator (Eagle Owl, *Bubo bubo*) and/or a lower habitat quality in the German territories, might have influenced the survival of adults in that population. However, it is very interesting how the Dutch population density remained quite stable in the two decades, whereas the German population had a steep growth in about the same period with such a lower adult survival rate.

In our population I found that neither annual reproductive success nor annual reproductive productivity were related to morph, but for the German population no comparative data have been published about individual morphs. In both populations, the long-term reproductive measures (cumulative and lifetime reproductive success) were morph dependent, favouring the intermediates, but the effect sizes were much larger in the German population. In our population I found that cumulative reproductive success was about 15% larger for the intermediates, whereas in the German population intermediates produced at least twice as many fledglings during their lives compared to dark or light morphs (Boerner and Krüger 2009) (table 6.1).

In our population I observed positive assortative mating with respect to plumage colour, with similar correlation coefficients for coloration scored on both the three-morph scale and the seven-morph scale (unpublished data). Moreover, I found that assortative pairs were more likely to produce offspring compared to disassortative pairs, and they formed a more stable pair over the years. In the German study, Krüger and colleagues were expecting to observe disassortative mating, being this mating pattern more adaptive to produce intermediate offspring in a population where intermediately coloured individuals have a fitness advantage (Krüger et al. 2001). Instead, they found that assortative mating occurs and the authors suggested it thus being maladaptive. However, apart from preference for same morph, Krüger et al (2001) tested other mate choice patterns but only from the female perspective, among which random mating and preference for the morph of the mother (based on expected probabilities of a given mother-morph from a simple Mendelian inheritance pattern). Despite these patterns all yield a similar good fit with the observed data, the authors affirm that sexual imprinting on the mother morph is the most likely mechanism of mate choice in their population. The conclusions of Krüger et al. (2001) about maladaptation of assortative mating and the mechanism of mate choice rely on a simple Mendelian inheritance of morph. These conclusions are not consistent with what was found in our study (Kappers et al. 2018).

It remains unclear why assortative pairs in our population are performing better, but it might be related to behavioural compatibility or to habitat matching. This is the case of another polymorphic raptor, where pair-level fitness advantages seem to be related to behaviour complementarity. Tate et al. (2017) supported the idea that differential fitness, consequence of morph combination, may explain balanced polymorphism in Black sparrowhawks *Accipiter melanoleucus* in South-Africa. The authors found that neither morph had a specific advantage in terms of productivity or survival; however, they found that morph combination of adult pairs influenced productivity significantly, with mixed-pairs producing more offspring per year than pairs consisting of the same morph. Although this

refers to higher success of disassortative pairs instead of assortative pairs, it is an example that pair-level fitness advantages may play an important role in promoting and maintaining polymorphism and may be important for bird species which display bi-parental care like Common buzzards.

Over the 20 years of our study, I found that the proportion of intermediates increased in our population. This apparent evolutionary change (as morphs are highly heritable) did likely arise due to the observed fitness advantage of individual phenotypes, but likely also from fitness benefits of assortative mating. As assortative pairs were more successful in producing offspring than disassortative pairs and assortatively paired intermediates produce a higher percentage of intermediate offspring (74%) than by following a simple Mendelian inheritance system (50%) (Kappers et al. 2018), this could lead to a further decline in frequencies of extreme phenotypes.

Krüger et al (2001) suggested that the fitness differences among individual morphs were the result of intermediate morphs breeding in highest quality territories (e.g. forested patches with functional nests that have high occupancy rate), and dark and light individuals also having a lower breeding propensity. Hence, they suggested that the competitive advantage of intermediate morphs (Krüger 2002), in combination with the large variation in territory quality resulted in the observed fitness advantage.

Dispersal behaviour in early life

If morphs differ in competitive abilities, it is important to understand different aspects of behaviour in young birds, such as degree of survival and the ability to obtain a breeding territory. In my work (Kappers et al., unpublished), I looked at natal dispersal of juveniles to try to unravel if morphs differ in these behavioural aspects during the early stage of their life. In several species, dispersers not only develop behavioural differences at the onset of dispersal, but display these behavioural characteristics through their life cycle (e.g. Howell et al. 2007). Personality-dependent dispersal is a phenomenon that can have important ecological consequences and it is relevant from an evolutionary point of view if correlated with melanic coloration.

I investigated if buzzard morphs in their early life have indeed different life-history strategies countering the selection against the fitness advantage of intermediate adult breeders in our population. I found that darker juveniles were more explorative than lighter morphs in the first months of the wandering stage, visiting few or several areas before settling. However, I did not find other significant differences among morphs, including emigration timing, distance travelled, and habitat choice. It remains unclear how this result could be interpreted in light of the fitness advantage of adult intermediates. A limitation is, that I only looked at the first months after emigration, and not at the whole dispersal process. This limitation was in part due to the devices itself, as they provide an incredible amount of detailed data but their activity can often be limited by the battery level (declining strongly in the dark winter months) and their overall lifetime. Therefore, I have no knowledge on how morphs may differ in the likelihood they have of obtaining a breeding territory. As buzzards take on average three to four years before starting to breed (Walls and

Kenward 2020), one future purpose could be to look at the data of the surviving juveniles for a longer period, even when the sample size is quite small because of mortality.

Territory quality seems less variable in our study area when compared to the German area (based on observed high occupancy rates, unpublished data). This may explain why I found only small fitness differences among adult morphs. Habitat variation in The Netherlands may be relatively small compared to other areas of buzzards' distributional range, and this might be why I did not find differences in habitat choice among dispersing juvenile morphs.

Maintenance of variation?

It is intriguing that a fitness benefit for intermediate buzzard morphs was found in both populations of adult breeders, but that despite this fitness benefit and the potential for evolutionary change, these populations are still highly variable for this genetically determined trait. In our population there may not be selective advantages or evident differences in life-history strategies that maintain different morphs. Factors such as sexual selection (e.g. assortative mating, see Lank and Fraser 2002) may maintain multiple morphs within the population, because of a certain inheritance pattern that always produces all morphs, not requiring further explanations of striking fitness differences.

Fitness differences among morphs were also investigated by Briggs and colleagues (2010) in another raptor species of the genus Buteo, the Swainson's hawk *Buteo swainsoni*. This bird of prey, similarly to the Common buzzard, shows continuous colour variation and has been categorized in three morphs. The authors investigated 32 years of breeding data and found no evidence that intermediate individuals (presumed heterozygotes) or the extreme morphs had increased levels of any component of fitness examined (Briggs et al. 2011). Therefore, Briggs et al. (2011) excluded both frequency-dependent selection and heterozygote advantage as mechanisms maintaining the colour polymorphism in this species. Interestingly, when looking at the large-scale distribution of Swainson's hawk morphs in their breeding range in North America, Amar and colleagues found a clinal variation with respect to plumage coloration, likely associated with temperature and rainfall (Amar et al. 2019).

In polymorphic species of birds, clinal variation in plumage coloration is frequently observed. Among colour-polymorphic birds, at least 20% show a cline in the relative frequency of morphs (Galeotti et al. 2003). Adaptation to local conditions should be reflected in relative changes of morph frequency as local habitat or climate conditions select against inappropriate phenotypes, resulting in clines across large spatial scales. For species with large ranges, quantifying the presence or nature of a cline is hampered by the requirement to collect unbiased field data for many specimens across extensive geographical areas. Therefore, despite clinal variation being fairly common, it has only been empirically explored in few species, such as Black sparrowhawk (Amar et al. 2014), Barn owl *Tyto alba* (Antoniazza et al. 2010) and Bananaquit *Coereba flaveola* (MacColl and Stevenson 2003).

A possibility for the Common buzzard could be that there is spatial variation in selection pressures on colour morphs (Gillespie and Turelli 1989), and phenotype-habitat

matching (Edelaar et al. 2008) at the species level. There is evidence for clines in colour morphs over large (Antoniazza et al. 2010; Amar et al. 2019) and smaller (Amar et al. 2014; Sordahl 2014) spatial scales in raptors, although there is relatively little evidence for a morph-by-habitat interaction on fitness (Dreiss et al. 2012). For my study species, remarkably little is known about the geographical distribution of the morphs (Ulfstrand 1977). Therefore, as part of the initial idea of my thesis I launched the "Buteo Morph" project where citizen scientists could 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. Preliminary and unpublished data seem to show clinal variation in morph frequencies of Common buzzards (see figure 6.1), confirming anecdotal information about the presence of higher proportions of darker morphs in the south and increasing frequencies of lighter morphs in the north-west across their breeding range in Europe.

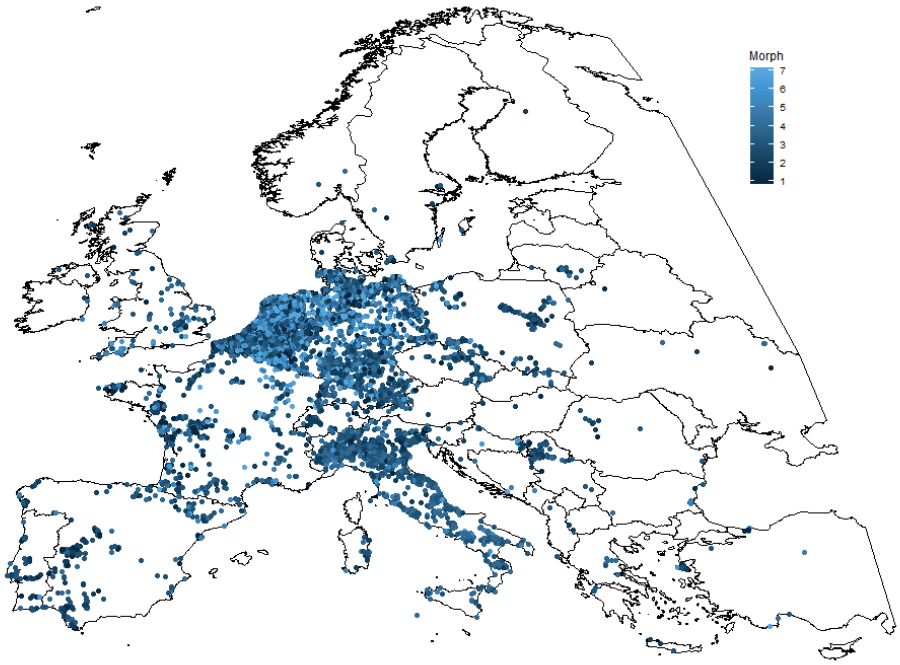

Figure 6.1: Raw data on the distribution of Common buzzard morphs collected by citizen scientists for the project Buteo-Morph from mid-2015 to mid-2017. Blue gradient corresponds to variation from darker morphs (1) to lighter morphs (7).

However, I did not investigate yet whether environmental factors may drive the spatial structuring of morphs in the species' range, and thus future research is needed.

This would surely fill the gap for the large-scale variation, but would still not explain why on the small scale – i.e. in one study population – we find such notable variation. What I missed to test in this thesis, is whether there was a phenotype-habitat matching in our

breeding population that could help us understand the presence of different morphs, even when intermediates seem to have an advantage over the years. I was able to look at habitat choice for juveniles during their first months of dispersal, but local habitat choice for breeding adults might also be very important to consider. A fitness advantage in adults could also be counter balanced by a different fitness trend in juveniles until their first breeding attempt, that unfortunately I could not completely quantify. When looking at first-time breeders in our population, we see that immigration of different morphs does not seem to be very structured over the years (figure 4.5c-d). Unfortunately, it was difficult to get data on whether the new breeders are immigrants from other populations or morphs recruited from ours. Worth to consider is that, as we found assortative mating, new immigrants might also be constrained by which morph is looking for a new mate to keep holding the territory in a certain season. Although I have been lucky enough to work with an incredible dataset, for a long-lived species like the common buzzard - that can live a couple of decades - it is risky to affirm that there is natural selection in place. For example, I did not have lifetime data for about 40% of the individuals, and of these, about 13% have been observed for 15 years out of the 20 of the study. We cannot rule out that there might be a positive frequency-dependent selection acting on our population, but we would need to keep monitoring the morphs for longer time to see evolutionary changes.

My thesis clearly highlights that understanding of evolutionary dynamics in natural populations requires not just a long-term effort in monitoring a focal population, but also needs to include all possible fitness consequences that may often accrue outside the specific study site (dispersal and habitat choice, spatial variation in fitness consequences on the smaller and larger scale).

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Samenvatting

De rijke verscheidenheid aan morfologische en gedragsmatige eigenschappen in de natuurlijke wereld komt voort uit de evolutionaire geschiedenis van soorten en populaties. Zichtbare fenotypische variaties binnen een soort (polymorfismen genoemd) zijn wijdverspreid in planten en dieren. Polymorfismen zijn interessant omdat ze erfelijk zijn en hierdoor uitstekend geschikt zijn als modelsysteem om micro-evolutionaire processen te onderzoeken. Fitness is een belangrijp concept binnen de evolutietheorie en is een maat voor de bijdrage aan de genenpool van een populatie door organismen. Door middel van fitness kunnen biologen natuurlijke selectie en micro-evolutie meten. In de natuur lijken morfen die met relatief stabiele frequenties naast elkaar bestaan vaak voor te komen. Persistent verenkleedpolymorfisme komt voor bij ongeveer 3,5% van de vogelsoorten, waarbij roofvogels een onevenredig hoge frequentie van dergelijke polymorfismen vertonen. Toch worden de mechanismen die ten grondslag liggen aan de evolutie en het behoud van polymorfismen in de natuur vaak slecht begrepen.

Het doel van dit proefschrift was het onderzoeken van de evolutionaire ecologie van kleurvariatie in een vogelsoort. Voor dit onderzoek hebben we de individuele fitness gekwantificeerd van een roofvogel met een zeer variabele verenkleed, de buizerd *Buteo buteo*. Hierbij hebben we enkele mechanismen geprobeerd te ontrafelen die de intra-specifieke kleurvariatie en de functies ervan in deze soort in stand houden. Daarnaast hebben we het kleurpolymorfisme bestudeerd zowel vanuit een tijds- als een ruimtelijk perspectief.

Om te begrijpen hoe het verenkleedpolymorfisme bij buizerds in stand wordt gehouden, is veel basiskennis nodig over het kleurenpolymorfisme zelf. Daarom hebben we eerst het type polymorfisme van de buizerd beschreven (hoofdstuk 2). We hebben de kleurvariatie van buizerds, zowel kwalitatief als kwantitatief, onderzocht en geprobeerd vast te stellen of het polymorfisme bij deze soort het beste gekwantificeerd kan worden als een discrete of continue eigenschap. Hiervoor hebben we gebruik gemaakt van digitaal fotomateriaal en pixelkleuring om de variatie te kwantificeren. We hebben aangetoond dat in buizerds de variatie continu en unimodaal is, variërend van zeer donkere tot zeer lichte individuen. Om onze resultaten te kunnen vergelijken met de gepubliceerde literatuur, hebben we de scoresystemen van onze en vorige studies op elkaar afgestemd. Tot slot hebben we onderzocht of het verenkleedpatroon van een individu gedurende het leven varieert, door de morf van individuen te scoren die gedurende meerdere jaren waren gefotografeerd. We vonden dat hoewel het verenkleed van de jonge tot de volwassen leeftijd iets donkerder werd, het morfotype niet wezenlijk veranderde.

Balanceringsselectie is een belangrijk mechanisme om de kleurpoly-morfismen in de loop van de evolutionaire tijd in stand te houden. Bij buizerds werd de variatie in de kleur van het verenkleed naar verluidt gehandhaafd door een heterozygote voordeel: heterozygote intermediaire morfen hadden een hogere fitness dan homozygote lichte en donkere morfen. We hebben een van de basisprincipes van de heterozygootvoordeel-hypothese op de proef gesteld, door te testen of de variatie in verenkleedkleur bij buizerds

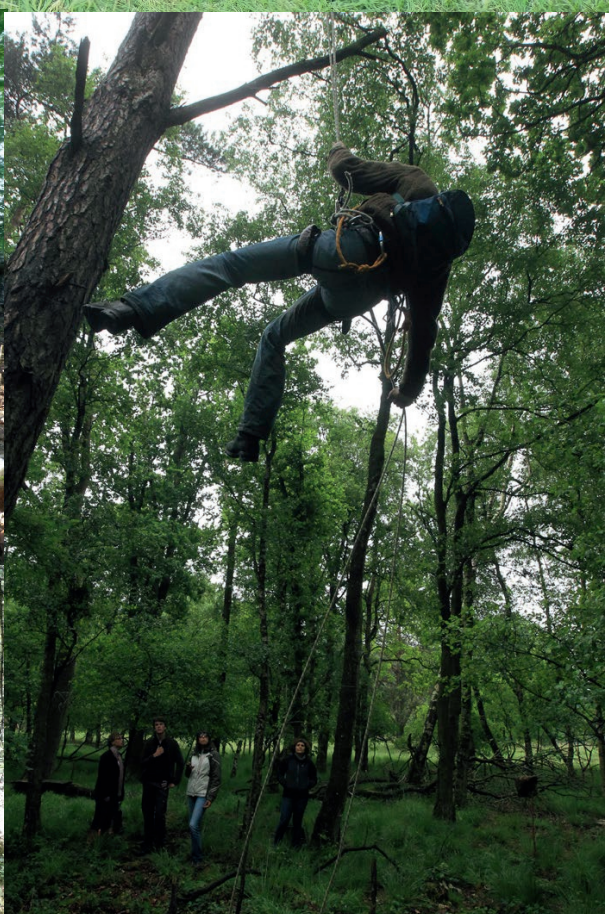
een een-locus twee-allelen overervingsmodel volgt (hoofdstuk 3). Met behulp van sociale stamboomgegevens uit het wild, met jonge vogels met bekende oudermorfen, hebben mijn collega's en ik de veronderstelde genetische basis van het kenmerk bevestigd. We hebben hiermee aangetoond dat kleurvariatie bij buizerds zeer erfelijk is. We vonden echter geen onderbouwing voor een eenvoudig Mendelian een-locus twee-allelen overervingsmodel. Onze resultaten suggereren dat de kleur van het verenkleed van buizerds als een kwantitatief polygene eigenschap moet worden beschouwd.

Gebruikmakend van 20 jaar aan broedgegevens hebben we eerdere studies naar de gevolgen van fitness van kleurpolymorfisme bij deze soort herhaald (hoofdstuk 4). We hebben morfeverschillen onderzocht in volwassen overleving, broedsucces, jaarlijks aantal geproduceerde jongen en cumulatief reproductief ssucces. We vonden dat de fitness verschilde tussen de morfen, waarbij de intermediaire morf de hoogste fitness had. Daarnaast werden assortatieve paringen voor kleurmorf waargenomen en vonden we dat assortatieve paren meer kans hadden om nakomelingen te produceren met langere paarbindingen dan disassortatieve paren. Bovendien, hebben we in onze lange termijn studie een fenotypische verandering gevonden met een toenemend aantal intermediaire morfen.

Hoewel de effecten van kleurvariatie voor verschillende levensgeschiedenis-kenmerken goed beschreven zijn, zijn de effecten op het dispersie-gedrag van de dieren onderbelicht. Aangezien de effecten van kleurpolymorfisme op de strategieën voor ruimtegebruik door de buizerd in de vroege stadia van het leven ontbreken, hebben we de effecten van de kleur van het verenkleed op het dispersiegedrag van buizerdjongen bestudeerd (hoofdstuk 5). Verder hebben we het effect van kleuring op de habitatkeuze bestudeerd in de eerste maanden van de zwerftocht. Met behulp van GPS-zenderdata verzameld in een Nederlandse populatie, hebben we getest of de kleur van het verenkleed invloed heeft op de emigratietijd, het aantal bezochte gebieden, de verblijfsduur in de gebieden, de cumulatieve afstand tussen de gebieden, de afstand tussen het gebied in de eerste winter en het nest en het aandeel van het gekozen bosrijke leefgebied. We vonden dat de kleuring alleen verband hield met het aantal bezochte gebieden, maar niet met andere kenmerken. Donkere individuen bezochten een groter aantal gebieden tijdens de eerste maanden van de dispersie in vergelijking met lichtere individuen.

Het proefschrift eindigt met een algemene discussie over de implicaties van onze bevindingen en toekomstperspectief (hoofdstuk 6).


Mûzebiterspaad



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And here I am, trying to condense many memories of a long period of time in a short deadline. But I have a good excuse: a move and the start of a new job in one of the strangest circumstances of the last few years... I recently moved back again to Groningen for a third phase of my life: right here, where the reason for the start of my PhD resides. From the first time I arrived for my master thesis in 2012, I found a special welcome and a very stimulating atmosphere in the Conservation Ecology group (at that time Animal Ecology group). Reasons why I wanted to come back...

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List of co-authors

Anneke Alberda[5]

Christiaan Both[1]

Nayden Chakarov[2,4]

Christiaan de Vries[5]

Wolfgang Forstmeier[3]

Bart Kempenaers[3]

Oliver Krüger[2]

Sylvia Kuhn[3]

Anna-Katharina Mueller[2]

Mihai Valcu[3]

[1]Conservation Ecology Group, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, the Netherlands;

[2]Department of Animal Behaviour, Bielefeld University, Bielefeld, Germany;

[3]Department of Behavioural Ecology and Evolutionary Genetics, Max Planck Institute for Ornithology, Seewiesen, Germany;

[4]Molecular Ecology and Evolution Lab, Lund University, Lund, Sweden;

[5]Noormanstrjitte, Wijnjewoude, The Netherlands.

About the author

Elena was born on the 6th of September, 1988 in Apeldoorn. After three years living in The Netherlands she moved to Italy with her parents Anna and Bert and her brother Martin. She grew up in Ciciliano, and then in Rome, where in 2007 she graduated from the classical lyceum Tacito. She obtained her BSc degree in Biology and her MSc degree in Ecology, both cum laude, at La Sapienza, University of Rome. In 2012, for her MSc thesis she travelled to The Netherlands as a "Free mover" to work at the University of Groningen on the effects of climate change on nestlings' diet in a migratory passerine, the Pied Flycatcher. There, she had the opportunity to do fieldwork for the first time and interact with a whole group of scientists all studying different bird species, and her interest for ecology and ornithology grew further. In 2013, she spent a year in central Italy working on Collared Flycatchers and broadening her fieldwork experience by taking part at different ringing projects. After that, in 2014 she was offered to come back to Groningen to start a PhD trajectory in the Conservation Ecology group with Christiaan Both and at the Max Planck Institute for Ornithology with Bart Kempenaers, the results of which can be read in this book. In October 2020, after some years living in Germany, she came back to Groningen again, to start as an ecologist consultant at Altenburg & Wymenga ecologisch onderzoek.

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