Communications

Journal of Avian Biology 47: 443–447, 2016
doi: 10.1111/jav.00794

Subject Editor: Peter Arcese. Editor-in-Chief: Jan-Åke Nilsson. Accepted 13 October 2015

Genetic markers validate using the natural phenotypic characteristics of shed feathers to identify individual northern goshawks *Accipiter gentilis*

Sarah R. Hoy, Rachel E. Ball, Xavier Lambin, D. Philip Whitfield and Michael Marquiss

S. R. Hoy (sarah.r.hoy@gmail.com), R. E. Ball, X. Lambin and M. Marquiss, School of Biological Sciences, Univ. of Aberdeen, Aberdeen AB24 2TZ, UK. – D. P. Whitfield, Natural Research Limited, Banchory, AB31 4BY, UK.

The recognition of individual animals is essential for many types of ecological research, as it enables estimates of demographic parameters such as population size, survival and reproductive rates. A popular method of visually identifying individuals uses natural variations in spot, stripe or scar markings. Although several studies have assessed the accuracy of these methods in mammals, crustaceans and fish, there have been few attempts to determine whether phenotypic characteristics are accurate when used for birds. Furthermore, even less is known about whether shed or moulted body parts can be reliably used to visually identify individuals. Here we assessed the accuracy of using phenotypic characteristics to identify avian individuals using a double-marking experiment, whereby nine microsatellite genetic markers and natural markings on shed feathers were used to independently identify northern goshawks *Accipiter gentilis*. Phenotypic and genetic identification of individuals was consistent in 94.4% (51/54) comparisons. Our results suggest that the phenotypic characteristics of shed feathers can be reliably used as a non-invasive and relatively inexpensive technique to monitor populations of an elusive species, the northern goshawk, without having to physically re-capture or re-sight individuals. We posit that using natural markings on shed feathers will also be a reliable method of identifying individuals in avian species with similar phenotypic characteristics, such as other *Accipiter* species.

Many areas of ecological and conservation research require individuals to be uniquely identifiable so that population sizes, dispersal, survival, reproduction and immigration rates can be estimated (Goodall 1986, Nichols 1992) and also for behavioural studies (Grellier et al. 2003, Weir 2009). Individuals can be made recognisable by applying various types of artificial marks or tags. However, the process of capturing individuals and applying such marks can be invasive, expensive, risky, time consuming and can affect the behaviour of the marked individual and its survival probability (reviewed by Walker et al. 2012). A less invasive method uses natural variation in phenotypic characteristics, such as stripe, spot or scar patterns to identify individuals (Pennycuick 1978, Goodall 1986, Friday and Smith 2000). Photographs of natural markings taken by camera traps is a particularly important method of identifying individuals in studies on large predators, whose wide-ranging and elusive behaviour makes it difficult to gather re-capture data or re-sighting data by eye (Trolle and Kéry 2003, Karanth et al. 2006, Ariefiandy et al. 2013). Although the use of natural markings and camera traps to collect photo-ID re-sighting data works well for some species, for several practical reasons the use of camera traps is rarely used in avian studies requiring the identification of individuals.

The phenotypic characteristics of moulted feathers have been used to identify individuals of elusive bird, without having to physically recapture or re-sight them by eye, or using camera traps. For example, natural markings on feathers moulted by several *Accipiter* species are thought to be stable (i.e. do not change over an individual's lifespan after the first moult) and vary enough between individuals to enable individuals to be identified, once in adult plumage (Opdam and Muskens 1976). However, of 19 studies using natural markings on shed feathers to identify individuals in *Accipiter* populations attempted to validate the method independently (for example see Rutz 2012, Saga and Selås 2012). It is important to assess the accuracy of methods used to identify individuals because such individual identities are often subsequently used and relied upon in a wide range of studies, across several disciplines. For example, individual identities are used to develop and evaluate conservation management strategies for tigers *Panthera tigris* (Karanth et al. 2006). The probability of incorrectly identifying two

individuals as the same (a false positive error) or of classifying two individuals as different, when in fact they are the same (a false negative error) has been long recognised (Bateson 1977). Yet, there have been relatively few attempts to validate the use of natural markings to identify individuals or to calculate the associated error rates (Stevick et al. 2001, Gosselin et al. 2007, Gubili et al. 2009, Waye 2013). Error rates can vary dramatically. For example, natural variation in pigmentation and scars correctly identified individual humpback whales *Megaptera novaeangliae* (Stevick et al. 2001) in 96.6% of cases; however the method of using colour and spot patterns to identify tiger salamanders *Ambystoma tigrinum* was only accurate 67% of the time (Waye 2013). This 10-fold variation in error rates, from excellent to effectively useless suggests a strong need to validate the different types of phenotypic characteristics used to identify individuals, for each taxonomic group, before they are used to estimate demographic parameters.

'Double marking', the use of two independent methods of identifying individuals, has been used to test the reliability of phenotypic characteristics as individual identifiers (Stevick et al. 2001, Gosselin et al. 2007, Gubili et al. 2009). However, we are only aware of one study which attempted to validate the use of phenotypic characteristics to identify individuals in an avian species using a double marking approach, which was based on comparisons of only five individuals sighted in two different years (Bretagnolle et al. 1994). Furthermore, there have been few studies which have used double marking to validate the use of shed body parts for individual identification (Gosselin et al. 2007). Northern goshawk *Accipiter gentilis* (hereafter goshawk) is an elusive avian predator, difficult to observe in wooded habitat and adults are difficult and time-consuming to physically capture. Although many studies have used natural markings on shed feathers to identify individual goshawks (e.g. Rutz 2012), none have attempted to validate the method independently. Microsatellites are neutral genetic markers used to identify individuals (Chistiakov et al. 2006) and have been used as an independent, unbiased and individually-fixed arbiter of the accuracy of phenotypic characteristics in double marking studies on cetaceans, crustaceans and fish (Stevick et al. 2001, Gosselin et al. 2007, Gubili et al. 2009). Five microsatellite markers have already been shown to uniquely identify individuals using blood samples taken from known individual northern goshawks (Bayard de Volo et al. 2005). Here we use nine microsatellite markers to genetically characterise, and if possible, identify individual goshawks from a population in north east Scotland, UK and use this method to assess the accuracy of using phenotypic characteristics of shed feathers as an identification tool.

Methods

Feather collection and phenotypic identification

Female goshawks start moulting their flight feathers during the egg laying period, whereas males are typically in moult between June–October, after the bulk of provisioning for offspring has been completed (Squires and Reynolds 1997). During the incubation period (April–May) many of inner primary feathers shed by females (and a few from males) can be found by searching below occupied nests and nearby perches. Shed feathers were collected from a goshawk population in north east Scotland, centred on 57°3′N, 2°30′W (map p. 138 in Marquiss 2011) and stored at room temperature in paper envelopes filed according to locality, year and date of collection.

The present study used only the inner primary feathers moulted by adult breeding female goshawks, as the majority of feathers located near active nests were those from females. The total feather length and width of calamus was used to sex the individual it came from, as comparable wing feathers moulted by male and female goshawks differ in size, with female feathers being larger (Cieslak and Dul 2006). The shape of each feather was used to determine which particular part of the wing sequence it was from (i.e. P1 to P5). Only feathers shed by mature individuals were included because the colour changes during the transition from immature (1 yr old) to mature (over 2 yr old) plumage (Opdam and Muskens 1976). The feathers of immatures are those grown simultaneously in the nest and are brown, fringed with buff, whilst those of mature birds (produced in sequence from the first moult) are plain grey, some with pale fringes; clearly different from those of yearlings (Cieslak and Dul 2006). To visually identify adult individuals we compared feathers from the same wing and position within the primary sequence, from year to year (e.g. P2 illustrated in Fig. 1, 2) using three phenotypic characteristics; length, colour and pattern of pigmentation as described in Opdam and Muskens (1976).

Genetic identification

DNA was extracted from a 3–5 mm clipping from the tip of the lower calamus, using a standard salt extraction protocol with a 100% ethanol precipitation following the methods in Hogan et al. (2008). All samples were genotyped at nine microsatellite loci, seven of which, Age2; Age4; Age5; Age7; Age9; Age10 and Age11 are described in Dawnay et al. (2009), and the remaining two, AgCA224 and AgCA365, in Takaki et al. (2008). These particular loci were chosen to maximise power for individual identity, as they were the most polymorphic microsatellite markers. PCR amplifications were performed in a 10 µl total reaction volume containing: 2 µl of extracted DNA, 1 × reaction Buffer (Bioline), 0.2 mM of each dNTP, 0.25 U *Taq* DNA polymerase (Bioline), 1.5 mM $MgCl_2$ and 1 µM primer using a G-Storm thermal cycler. Genotypes were resolved on an automatic ABI 3730 Capillary DNA sequencer (DNA Sequencing and Services, MRCPPU, College of Life Sciences, Univ. of Dundee, Scotland, <www.dnaseq.co.uk>). Allele size was determined by eye using Genemarker 1.4 (Soft Genetics).

We checked all genotyping scores for errors resulting from the presence of null alleles (one or more alleles failing to amplify), stuttering (changes to allele sizes during PCR) and large allele drop out (large alleles not amplifying as efficiently as smaller alleles) using Microchecker 2.2.1 (Van Oosterhout et al. 2004). The rate of genotyping error was estimated by re-genotyping eight samples (9% of the data) at all loci and error rates were calculated from the number of allelic mismatches.

We calculated the probability of individual identity, *P*(ID) as the probability that two individuals, drawn at random from a population will share the same genotypic

Figure 1. Dorsal side of moulted female *Accipiter gentilis* inner primary feathers collected at the same nest site location, in subsequent years, assigned as belonging to the same individual based on their phenotypic characteristics (length, shape, colour and pattern of pigmentation).

Figure 2. Dorsal side of four female *Accipiter gentilis* inner primary feathers collected at the same nest site in different years, thought to have been moulted by different individuals based on their phenotypic characteristics (length, shape, colour and pattern of pigmentation).

profile, according to Waits et al. (2001) for all nine loci using Genalex 6.501 (Peakall and Smouse 2006). $P(\mathrm{ID})_{\mathrm{sib}}$ represents the upper boundary of $P(\mathrm{ID})$ (where siblings are found and included; Waits et al. 2001) and $P(\mathrm{ID})_{\mathrm{unbiased}}$ represents the lower boundary of a theoretical $P(\mathrm{ID})$, after sample size corrections (Paetkau et al. 1998). We included both boundaries as the true $P(\mathrm{ID})$ has been demonstrated to fall somewhere between these two, with $P(\mathrm{ID})_{\mathrm{sib}}$ providing a reliable conservative estimate of the upper boundary, assuming that the population studied does not deviate from Hardy–Weinberg expectations (Waits et al. 2001). Departures from Hardy–Weinberg equilibrium (HWE) were tested for using a Markov Chain Monte Carlo approach (1000 de-memorisations, 100 batches, 1000 iterations) in GENEPOP 4.0.10 (Raymond and Rousset 1995, Rousset 2008) incorporating a Bonferroni correction ($\alpha = 0.005$).

Validation of phenotypic method

We used 83 feathers collected over a 15 yr period, from 26 nesting territories occupied by goshawks. We compared feathers collected at the same location, but in different years, as goshawks in the UK are resident, persistently use the same nesting woods and only breed once a year, hence are unlikely to be represented at multiple sites in the same year (Kenward 2006). Phenotypic identification was carried out by MM using the measures described by Opdam and Muskens (1976) and preceded genetic identification, carried out by RB. Both methods were applied independently and as a double blind test to reduce any potential bias. No results were exchanged until after the genetic analysis was complete.

Results and discussion

We were able to genotype 98.8% (82/83) of our feather samples. Aside from a failed amplification, there were no genotyping errors and we did not detect any null alleles or large allelic dropout at any locus. Between 2 and 13 alleles were scored per locus, with $P(\mathrm{ID})_{\mathrm{unbiased}}$ for all loci estimated as 5.8×10^{-8} and $P(\mathrm{ID})_{\mathrm{sib}}$ as 1.1×10^{-3} (Table 1), meaning that the probability of two individuals sharing the same multilocus profile was less than 0.0001. Genetic markers suggested

Table 1. Probability of identity estimates for nine microsatellite markers for *A. gentilis*. N_A number of alleles, * cumulative values for $P(\mathrm{ID})$.

Locus	N_A	$P(\mathrm{ID})_{\mathrm{unbiased}}$	$P(\mathrm{ID})_{\mathrm{sib}}$	$P(\mathrm{ID})_{\mathrm{unbiased}}$*	$P(\mathrm{ID})_{\mathrm{sib}}$ *
Age 2	10	5.8×10^{-2}	3.6×10^{-1}	5.8×10^{-2}	3.6×10^{-1}
Age 4	13	4.2×10^{-2}	3.4×10^{-1}	2.4×10^{-3}	1.2×10^{-1}
Age 5	5	3.3×10^{-1}	6.0×10^{-1}	8.0×10^{-4}	7.1×10^{-2}
Age 7	5	1.3×10^{-1}	4.2×10^{-1}	1.0×10^{-4}	3.0×10^{-2}
Age 9	2	3.9×10^{-1}	6.1×10^{-1}	4.2×10^{-5}	1.8×10^{-2}
Age 10	8	1.0×10^{-1}	4.2×10^{-1}	4.5×10^{-6}	7.8×10^{-3}
Age 11	4	2.4×10^{-1}	5.3×10^{-1}	1.1×10^{-6}	4.2×10^{-3}
AG CA224	4	2.7×10^{-1}	5.5×10^{-1}	3.1×10^{-7}	2.3×10^{-3}
AG CA365	5	1.8×10^{-1}	4.8×10^{-1}	5.8×10^{-8}	1.1×10^{-3}

that these 82 samples came from 37 unique individuals. Of the 54 comparisons made between pairs of samples collected at the same goshawk nest territory, 36 were phenotypically identified as being samples from the same individual (see Fig. 1 for an example); the remaining 18 comparisons were phenotypically identified as being samples from different individuals (see Fig. 2 for an example). Phenotype-based and genetic assignments of individuals matched in 51 (94.4%) comparisons. Of the three discrepancies found, one was a false positive (i.e. two samples were thought to have come from the same individual based on phenotypic characteristics, but were genetically assigned as different individuals); the other two discrepancies were false negatives (i.e. where two samples were phenotypically identified as coming from different individuals, yet were from genetically identical individuals). The false positive and false negative error rates were therefore 97.2 and 88.9% respectively. The phenotypic method of identifying individual goshawks from shed feathers described by Opdam and Muskens (1976) therefore appears to be reliable. Consequently, despite goshawks being elusive, changes in the individuals occupying nest sites can be reasonably accurately monitored using this relatively inexpensive phenotypic technique, without further recourse to genotyping. Analysis of avian vocalizations can also be used as an alternative, relatively inexpensive way to identify individuals occupying territories, without having to collect moulted feathers, genetic, re-sighting or re-capture data. However the reliability of this method has yet to be assessed for the majority of bird of prey species, with the exception of a few owl species' (Tripp and Otter 2006, Nagy and Rockwell 2012, Odom et al. 2013).

Overall our results suggest that phenotypic characteristics of shed feathers are a reliable method of identifying individual goshawks, and may be similarly accurate for other species, such as Eurasian sparrowhawks *Accipiter nisus,* thought to show a similar level of variation in feather characteristics (Opdam and Muskens 1976). Furthermore, now that the error rate of using the phenotypic method has been quantified, it can be accounted for in future studies using this phenotypic method and when evaluating the status of populations and planning management strategies. These error rates may also be used to calculate the degree of confidence one can have when interpreting the results of previous studies using this phenotypic method.

Acknowledgements – We are grateful to S. Piertney for allowing access to laboratory facilities and to M. Wenzel, R. Ogden and G. Murray-Dickson for their advice on genetic methods. This research was partly funded by a Natural Environment Research Council studentship NE/J500148/1 to SH and by Natural Research Limited.

References

Ariefiandy, A., Purwandana, D., Seno, A., Ciofi, C. and Jessop, T. S. 2013. Can camera traps monitor komodo dragons a large ectothermic predator? – PLoS One 8: e58800.

Bateson, P. P. G. 1977. Testing an observer's ability to identify individual animals. – Anim. Behav. 25: 247–248.

Bayard de Volo, S., Reynolds, R. T., Topinka, J. R., May, B. and Antolin, M. F. 2005. Population genetics and genotyping for mark–recapture studies of northern goshawks (*Accipiter gentilis*) on the Kaibab Plateau, Arizona. – J. Raptor Res. 39: 286–295.

Bretagnolle, V., Thibault, J. C. and Dominici, J. M. 1994. Field identification of individual ospreys using head marking patterns. – J. Wildl. Manage. 58: 175–178.

Chistiakov, D. A., Hellemans, B. and Volckaert, F. A. M. 2006. Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. – Aquaculture 255: 1–29.

Cieslak, M. and Dul, B. 2006. Feathers: identification for bird conservation. – Natura Publishing House.

Dawnay, N., Ogden, R., Wetton, J. H., Thorpe, R. S. and McEwing, R. 2009. Genetic data from 28 STR loci for forensic individual identification and parentage analyses in 6 bird of prey species. – Forensic Sci. Int. Genet. 3: 63–69.

Friday, N. and Smith, T. 2000. Measurement of photographic quality and individual distinctiveness for the photographic identification of humpback whales, *Megaptera novaeangliae*. – Mar. Mammal Sci. 16: 355–374.

Goodall, J. 1986. The chimpanzees of Gombe: patterns of behaviour. – The Belknap Press of Harvard Univ. Press.

Gosselin, T., Sainte-Marie, B. and Sevigny, J. M. 2007. Individual identification of decapod crustaceans II: natural and genetic markers in snow crab (*Chionoecetes opilio*). – J. Crustac. Biol. 27: 399–403.

Grellier, K., Hammond, P. S., Wilson, B., Sanders-Reed, C. A. and Thompson, P. M. 2003. Use of photo-identification data to quantify mother–calf association patterns in bottlenose dolphins. – Can. J. Zool. 81: 1421–1427.

Gubili, C., Johnson, R., Gennari, E., Oosthuizen, W. H., Kotze, D., Meÿer, M., Sims, D. W., Jones, C. S. and Noble, L. R. 2009. Concordance of genetic and fin photo identification in the great white shark, *Carcharodon carcharias*, off Mossel Bay, South Africa. – Mar. Biol. 156: 2199–2207.

Hogan, F. E., Cooke, R., Burridge, C. P. and Norman, J. A. 2008. Optimizing the use of shed feathers for genetic analysis. – Mol. Ecol. Resour. 8: 561–567.

Karanth, K. U., Nichols, J. D., Kumar, N. S. and Hines, J. E. 2006. Assessing tiger population dynamics using photographic capture–recapture sampling. – Ecology 87: 2925–2937.

Kenward, R. E. 2006. The goshawk. – T and A D Poyser.

Marquiss, M. 2011. Goshawk. – In: Francis, I. and Cook, M. (eds), The breeding birds of north east Scotland. Scottish Ornithologists' Club, pp. 138–139.

Nagy, C. M. and Rockwell, R. F. 2012. Identification of individual eastern screech-owls *Megascops asio* via vocalization analysis. – Bioacoustics 21: 127–140.

Nichols, J. D. 1992. Capture–recapture models: using marked animals to study population dynamics. – Bioscience 42: 94.

Odom, K. J., Slaght, J. C. and Gutiérrez, R. J. 2013. Distinctiveness in the territorial calls of great horned owls within and among years. – J. Raptor Res. 47: 21–30.

Opdam, P. and Muskens, G. 1976. Use of shed feathers in population studies of *Accipiter* hawks (Aves, Accipitriformes, Accipitridae). – Beaufortia 24: 55–62.

Paetkau, D., Waits, L. P., Clarkson, P. L., Craighead, L., Vyse, E., Ward, R. and Strobeck, C. 1998. Variation in genetic diversity across the range of North American brown bears. – Conserv. Biol. 12: 418–429.

Peakall, R. and Smouse, P. E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. – Mol. Ecol. Notes 6: 288–295.

Pennycuick, C. J. 1978. Identification using natural markings. – In: Stonehouse, B. (ed.), Animal markings. MacMillan, pp. 147–159.

Raymond, M. and Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. – J. Hered. 86: 288–295.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. – Mol. Ecol. Resour. 8: 103–106.

Rutz, C. 2012. Predator fitness increases with selectivity for odd prey. – Curr. Biol. 22: 820–824.

Saga, Ø. and Selås, V. 2012. Nest reuse by goshawks after timber harvesting: importance of distance to logging, remaining mature forest area and tree species composition. – For. Ecol. Manage. 270: 66–70.

Squires, J. R. and Reynolds, R. T. 1997. Northern goshawk (*Accipiter gentilis*). – In: Poole, A. and Gill, F. (eds), The birds of North America. Birds of North America, no. 298.

Stevick, P. T., Palsbøll, P. J., Smith, T. D., Bravington, M. V. and Hammond, P. S. 2001. Errors in identification using natural markings: rates, sources, and effects on capture–recapture estimates of abundance. – Can. J. Fish. Aquat. Sci. 58: 1861–1870.

Takaki, Y., Kawahara, T., Kitamura, H., Endo, K. and Kudo, T. 2008. Genetic diversity and genetic structure of northern goshawk (*Accipiter gentilis*) populations in eastern Japan and central Asia. – Conserv. Genet. 10: 269–279.

Tripp, T. M. and Otter, K. A. 2006. Vocal individuality as a potential long-term monitoring tool for western screech-owls, *Megascops kennicottii*. – Can. J. Zool. 84: 744–753.

Trolle, M. and Kéry, M. 2003. Estimation of ocelot density in the pantanal using capture–recapture analysis of camera-trapping data. – J. Mammal. 84: 607–614.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. – Mol. Ecol. Notes 4: 535–538.

Waits, L. P., Luikart, G. and Taberlet, P. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. – Mol. Ecol. 10: 249–256.

Walker, K. A., Trites, A. W., Haulena, M. and Weary, D. M. 2012. A review of the effects of different marking and tagging techniques on marine mammals. – Wildl. Res. 39: 15–30.

Waye, H. L. 2013. Can a tiger change it's spots? a test of the stability of spot patterns for identification of individual tiger salamanders (*Ambystoma tigrinum*). – Herpetol. Conserv. Biol. 8: 419–425.

Weir, C. R. 2009. Distribution, behaviour and photo-identification of Atlantic humpback dolphins *Sousa teuszii* off Flamingos, Angola. – Afr. J. Mar. Sci. 31: 319–331.