J Ornithol (2016) 157:919–922
DOI 10.1007/s10336-016-1354-2

SHORT COMMUNICATION

No genetic structure in a mixed flock of migratory and non-migratory Mallards

Robert H. S. Kraus[1,2] · Jordi Figuerola[3,4] · Katharina Klug[1,2]

Received: 16 February 2016 / Revised: 2 May 2016 / Accepted: 3 May 2016 / Published online: 12 May 2016

Abstract Mallards do not show genetic differentiation into migratory populations across typical flyways. It is also known that some Mallard populations are non-migratory. The aim of this study was to test if genetic structure exists between migratory and non-migratory Mallards in an area where they occur sympatrically, in Doñana, Spain. After quality filtering we analysed 350 single nucleotide polymorphism markers (SNPs) from 104 migratory and non-migratory Mallards. No genetic structure was evident from our data. We conclude that the lack of large-scale genetic structure of the global Mallard population remains valid when specifically testing potential differentiation between migratory and non-migratory Mallards.

Keywords Migration · Genetic assignment · Genetic structure · *Anas platyrhynchos* · Ducks · Waterfowl

Zusammenfassung

Keine genetische Struktur in einer gemischten Gruppe aus ziehenden und nicht-ziehenden Stockenten

Stockenten weisen keine genetische Differenzierung in typische Vogelzugrouten auf. Es ist auch bekannt, dass manche Stockentenpopulationen aus Standvögeln bestehen, die nicht ziehen. Das Ziel dieser Studie war die Untersuchung einer möglichen genetischen Differenzierung zwischen ziehenden und nicht-ziehenden Stockenten in einem Gebiet, in dem diese sympatrisch vorkommen, in Doñana, Spanien. Nach Qualitätsfilterung analysierten wir 350 Einzel-Nukleotid-Polymorphismen (single nucleotide polymorphisms; SNPs) von 104 ziehenden und stehenden Stockenten. Es gab keine Hinweise auf genetische Struktur in unseren Daten. Wir schließen daraus, dass das Fehlen genetischer Struktur in der globalen Stockentenpopulation auch dann noch zutrifft, wenn man lokal spezifisch ziehende und stehende Populationen vergleicht.

Migration in birds can be genetically determined or culturally learned. However, the performance of migratory behaviour can be subject to change, either by changes in the genetic background (e.g. Blackcaps; Mettler et al. 2013) or behavioural traditions (e.g. Barnacle Geese; Jonker et al. 2013). A migratory species consists of populations that consistently travel between their wintering and breeding grounds. But this textbook description does not always apply in reality. Especially in ducks it is common that flyways have no strict boundaries and switching between flyways could clearly be demonstrated e.g. through observations in Eurasian Teals (*Anas crecca*) (Guillemain et al. 2005) or indirectly through genetics in

Communicated by M. Wink.

✉ Robert H. S. Kraus
robert.kraus@uni-konstanz.de

[1] Department of Biology, University of Konstanz, 78457 Konstanz, Germany

[2] Department of Migration and Immuno-Ecology, Max Planck Institute for Ornithology, Am Obstberg 1, 78315 Radolfzell, Germany

[3] Estación Biológica de Doñana (CSIC), Av. Américo Vespucio, s/n., 41092 Seville, Spain

[4] CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

Mallards (*Anas platyrhynchos*) (Kraus et al. 2013). Additionally, Mallards are a species known to exhibit population variation in migratory behaviour i.e. some populations migrate while others do not (and there is individual flexibility, too). Their partial migration and frequent changes in migration routes cause a lack of global population structure in Mallards (Kraus et al. 2013). Locally, however, few studies investigated the genetics of migratory flexibility in Mallards. To our knowledge, the only study showing genetic differentiation among wild Mallards on a local scale is from Italy where genetic differences were detected between urban and rural populations as a result of urbanisation of wild populations (Baratti et al. 2014). It remains to be tested if such genetic differences also arise by different migratory strategies. The aim of this study was to analyse genetic structure in a mixed flock of migratory and non-migratory Mallards.

The Mallard samples used in this analysis were collected in Doñana, south-western Spain. A local non-migratory Mallard population breeds in the area, but many migratory birds use the national park as a stopover on the travel to their wintering grounds, or winter there directly (Navedo et al. 2012; Rendón et al. 2008). This area is a perfect site to study potential differences between sympatric migratory and non-migratory Mallards. Because we expected a weak genetic signal (if present at all) we employed a powerful set of 384 genome-wide single nucleotide polymorphism markers (SNPs; Kraus et al. 2011). Blood was collected from Mallards trapped throughout all seasons, such that our sample contained periods when only resident Mallards were present and also when migratory Mallards were more abundant. Genetic analyses were performed as described previously (Kraus et al. 2013). After quality filtering with a 10 % threshold for missing data across SNP loci and individuals, we retained a data set of 104 individuals (sampled between 2001 and 2010) with genotype information for 350 SNPs.

We tested for the existence of genetic structure by visualising the genetic distribution of genotypes in a principal coordinate analysis in GenAlEx 6.5 (Peakall and Smouse 2012). No clear patterns in similarity were found among vs. between seasons across all years when samples were available. A quantitative way of testing genotypic data for the presence of population structure is individual-based modelling with the software Structure (Pritchard et al. 2000). In Structure, we could evaluate models where we assumed the presence of $K = 1$–10 populations; each model (allele frequencies correlated, admixture model) tested across 10 replicate runs of 20,000 MCMC iterations of which the first 10 % were discarded as burn-in. The overall model score "Ln $P(K)$" showed no plateau across the ranges of K that would indicate the presence of a clear number of biologically relevant clusters (Fig. 1a). The objective ad hoc statistic ΔK for evaluating Structure output (Earl and vonHoldt 2011; Evanno et al. 2005) did not favour a particular partitioning of the individuals into one of the K clusters, either (Fig. 1b). The performance of Structure may be reduced by violations of its population genetic model assumptions (Jombart et al. 2010). We therefore employed an alternative clustering method that is free from such assumptions and only uses genetic similarities: discriminant analysis of principal components (DAPC; Jombart et al. 2010). We closely followed the DAPC manual to infer the number of clusters in the data and made use of the built-in function *optim.a.score* to avoid overfitting of our genetic cluster models. DAPC has an implemented measure to evaluate model fit, too, the Bayesian information criterion (BIC). The BIC curve across $K = 1$–10 clearly indicated $K = 1$ to be the best fitting model (Fig. 1c).

The previous evaluation of the genetic data with two complementary algorithms (Structure, DAPC) provided no indication of the presence of multiple genetic clusters in our sample of Mallards from Doñana. However, to fully scrutinise our data we forced Structure and DAPC to assign each individual Mallard to one of two clusters in a model of $K = 2$, i.e. a hypothesised migratory vs. a non-migratory cluster (Fig. 2). With a threshold of 0.85 probability we assigned 10 Mallards to cluster 1 and 11 to cluster 2. Eighty-three individuals could not be assigned under this threshold (numbers for Structure; DAPC provided similar assignments although it was more decisive in some cases for individuals of the unknown cluster; see also Söderquist et al. (2016). The clear assignment of 10 individuals to cluster 1 did not coincide with Mallards sampled in a certain season. To turn this approach around, we studied the ringing histories of the 104 Mallards in our data set and found that we could define 17 Mallards as locals (1 male, 16 females) while three individuals were clearly wintering Mallards (2 males, 1 female). A detailed examination of the genetic assignment profiles by Structure and DAPC of these individuals revealed that no pattern existed whether these all belong to a "migratory" or "sedentary" cluster.

It has been shown that genetic differences among wild Mallards can arise by the introduction of farm-raised Mallards. In many European countries there is an increasing tendency to restock local wild Mallard populations for the purpose of hunting. The individuals used for these restocking programmes stem from farms that breed wild-looking Mallards. It is possible that those individuals assigned to cluster 1 by the genetic clustering algorithms are released farm Mallards and consequently show a distinct genetic identity even though looking like wild Mallards. Söderquist et al. (2016) used the same SNP marker system as in this study and deciphered the genetic make-up

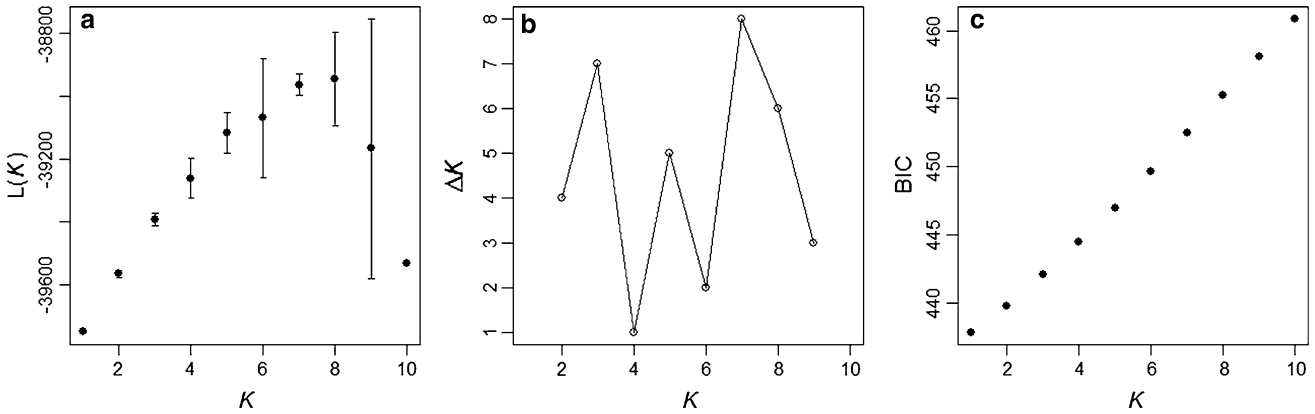

Fig. 1 The outcomes of STRUCTURE and DAPC analyses. **a** The raw mean model likelihood $L(K)$ of all ten replicate STRUCTURE runs for each model of K populations with their standard deviations. **b** The evaluation of each K by Evanno's ΔK statistic. No single solution for any K is obvious. **c** Bayesian information criterion (BIC) from DAPC for each number of clusters of $K = 1$–20. BIC values closest to zero indicate best fit of the data to a model

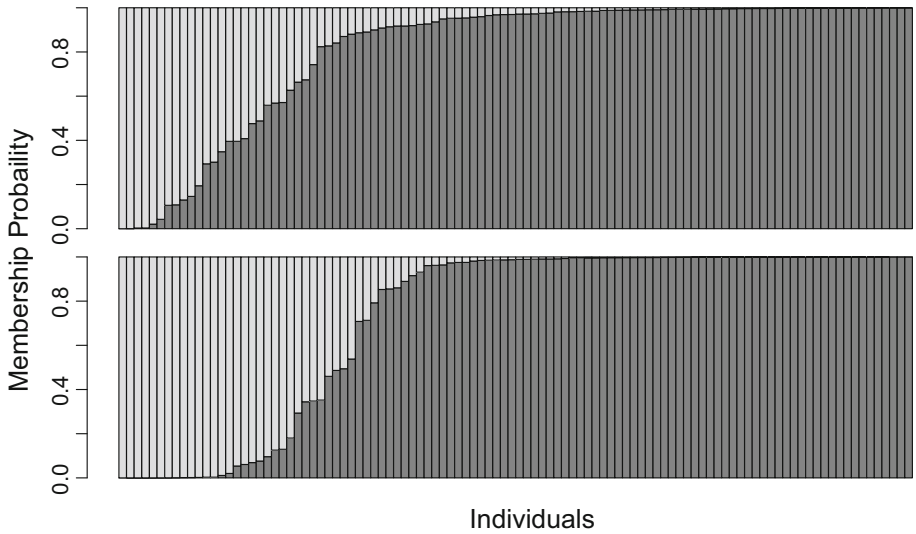

Fig. 2 Individual membership probabilities (from 0 to 1.0) of 104 Mallards in the programmes STRUCTURE (*top*) and DAPC (*bottom*) at $K = 2$. Each *bar* represents one individual. Individuals were sorted by their assignment probabilities in decreasing order to cluster 1 (*light grey*), with assignments to cluster 2 to the *right* (*dark grey*)

of farm vs. wild Mallards in several countries in Europe. They genotyped reference individuals from farms in three countries and were able to genetically discriminate between farm and wild Mallards. The reference farm Mallards that were geographically closest to Doñana were those collected in France. We included genetic profiles of these French farm Mallards from Söderquist et al. in one STRUCTURE analysis with the samples genotyped in this study. The results confirm that only two individuals of cluster 1 were assigned to Mallards of farm origin (data not shown). The remaining 102 individuals carried a clear wild mallard genotype in this analysis, and also in analyses with other reference data sets such as the full list of Söderquist et al.'s farm and wild Mallards, as well as the global Mallard data set of Kraus et al. (2013). We therefore exclude the possibility that cluster 1 potentially constitutes a subset of farmed Mallards.

A model with $K = 2$ (or larger K) is neither supported by STRUCTURE or DAPC, nor are the individual assignments in a forced $K = 2$ model meaningful in a biological way (ringing histories, potential farm origin). Our initial hypothesis was that migratory and non-migratory Mallards form distinct populations and that this should be reflected in their genotypes. Knowing about the lack of population genetic structure in Mallards on broad geographical scales (Kraus et al. 2013), we specifically chose the Doñana system to test this in a well-studied regional setting where both groups occur sympatrically. Our results were negative. Mallards sampled in Doñana across multiple years and through every season are genetically uniform. We are confident that Mallards of both migratory strategies were included in our sampling as we know that numbers fluctuate between summer and winter populations, and also because we could indeed identify some clear migrants and

non-migrants through evaluation of their ringing records. At first glance it appears obvious that no population structure was present among Doñana Mallards. Ducks are known to exhibit substantial "abmigration" in general (the switching between flyways; Guillemain et al. 2005) and the Mallard is no exception (Kraus et al. 2013). On the other hand, previous studies specifically targeting Mallard populations with special histories such as farming (Söderquist et al. 2016) or urbanisation (Baratti et al. 2014) indeed showed differentiation within small scales. We here provide the first attempt to study differentiation between migratory and non-migratory Mallards with a powerful genome-wide SNP set (Kraus et al. 2011) and follow multiple lines of evidence to arrive at our conclusion. Even though this is a negative result, the publication of negative results is of great importance to the scientific community and recent trends towards hiding such outcomes are worrisome (Fanelli 2012). The de facto panmictic population structure in wild Mallards does not only hold in a global context but also locally, like here in Doñana suggesting that gene flow or behavioural changes in migratory strategy are common enough to prevent population differentiation.

Acknowledgments The genotyping of samples in this study was performed in the Animal Breeding and Genomics Group (Wageningen, the Netherlands) with support from Martien Groenen, Richard Crooijmans and Bert Dibbits. The data of this manuscript is from a project in the Resource Ecology Group (Herbert Prins, Wageningen, the Netherlands) financed by the KNJV (Royal Netherlands Hunters Association), the Dutch Ministry of Agriculture, the Faunafonds, the Stichting de Eik Trust. Field work in Spain was supported by the MINECO project CGL2012-30759 and the EU projects EDEN and EDENEXT. Ringing data was obtained from "Oficina de Anillamiento", Madrid, Spain. Katharina Klug thanks Martin Wikelski for hosting her as a BSc student during the analyses. All work presented in this study complies with the laws of the country in which they were performed.

References

Baratti M, Baccetti N, Cordaro M, Mori A, Dessì-Fulgheri F (2014) Investigating the puzzling genetic structure of mallard populations (*Anas platyrhynchos* L.) in Italy. Eur J Wildl Res 61:81–89. doi:10.1007/s10344-014-0876-2

Earl DA, vonHoldt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Cons Gen Res 4:359–361. doi:10.1007/s12686-011-9548-7

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Fanelli D (2012) Negative results are disappearing from most disciplines and countries. Scientometrics 90:891–904. doi:10.1007/s11192-011-0494-7

Guillemain M, Sadoul N, Simon G (2005) European flyway permeability and abmigration in Teal *Anas crecca*, an analysis based on ringing recoveries. Ibis 147:688–696

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94

Jonker RM et al (2013) Genetic consequences of breaking migratory traditions in barnacle geese *Branta leucopsis*. Mol Ecol 22:5835–5847

Kraus RHS et al (2011) Genome wide SNP discovery, analysis and evaluation in mallard (*Anas platyrhynchos*). BMC Genom 12:150

Kraus RHS, Van Hooft P, Megens H-J, Tsvey A, Fokin SY, Ydenberg RC, Prins HHT (2013) Global lack of flyway structure in a cosmopolitan bird revealed by a genome wide survey of single nucleotide polymorphisms. Mol Ecol 22:41–55. doi:10.1111/mec.12098

Mettler R et al (2013) Contrasting patterns of genetic differentiation among blackcaps (*Sylvia atricapilla*) with divergent migratory orientations in Europe. PLoS One 8. doi:10.1371/journal.pone.0081365

Navedo JG et al (2012) International importance of Extremadura, Spain, for overwintering migratory dabbling ducks: a role for reservoirs. Bird Conserv Int 22:316–327. doi:10.1017/s0959270911000311

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539. doi:10.1093/bioinformatics/bts460

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Rendón MA, Green AJ, Aquilera E, Almaraz P (2008) Status, distribution and long-term changes in the waterbird community wintering in Doñana, south-west Spain. Biol Conserv 141:1371–1388. doi:10.1016/j.biocon.2008.03.006

Söderquist P et al (2016) Released game birds may cause continent-wide introgression: a changing genetic landscape in European mallard (*Anas platyrhynchos*) (**in press**)