A tree of geese: A phylogenomic perspective on the evolutionary history of True Geese

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A B S T R A C T

Phylogenetic incongruence can be caused by analytical shortcomings or can be the result of biological processes, such as hybridization, incomplete lineage sorting and gene duplication. Differentiation between these causes of incongruence is essential to unravel complex speciation and diversification events. The phylogeny of the True Geese (tribe Anserini, Anatidae, Anseriformes) was, until now, contentious, i.e., the phylogenetic relationships and the timing of divergence between the different goose species could not be fully resolved. We sequenced nineteen goose genomes (representing seventeen species of which three subspecies of the Brent Goose, *Branta bernicla*) and used an exon based phylogenomic approach (41,736 exons, representing 5887 genes) to unravel the evolutionary history of this bird group. We thereby provide general guidance on the combination of whole genome evolutionary analyses and analytical tools for such cases where previous attempts to resolve the phylogenetic history of several taxa could not be unravelled. Identical topologies were obtained using either a concatenation (based upon an alignment of 6,630,626 base pairs) or a coalescent based consensus method. Two major lineages, corresponding to the genera *Anser* and *Branta*, were strongly supported. Within the *Branta* lineage, the White cheeked Geese form a well supported sub lineage that is sister to the Red breasted Goose (*Branta ruficollis*). In addition, two main clades of *Anser* species could be identified, the White Geese and the Grey Geese. The results from the consensus method suggest that the diversification of the genus *Anser* is heavily influenced by rapid speciation and by hybridization, which may explain the failure of previous studies to resolve the phylogenetic relationships within this genus. The majority of speciation events took place in the late Pliocene and early Pleistocene (between 4 and 2 million years ago), conceivably driven by a global cooling trend that led to the establishment of a circumpolar tundra belt and the emergence of temperate grasslands. Our approach will be a fruitful strategy for resolving many other complex evolutionary histories at the level of genera, species, and subspecies.

Keywords:

Consensus

Concatenation

Gene tree

Hybridization

Incomplete lineage sorting

Species tree

1. Introduction

Incongruence between phylogenies generated from different sets of genetic and phenotypic data poses a significant challenge

in evolutionary biology. Such incongruence can be caused by analytical shortcomings (Davalos et al., 2012; Rokas et al., 2003), such as issues with limited taxon sampling, unmet assumptions in the modelling of sequence evolution, and the choice of different optimality criteria (Graybeal, 1998; Rokas et al., 2003; Yang et al., 1994). Phylogenetic incongruence can also be generated by biological processes, such as hybridization, incomplete lineage sorting and gene duplication (Degnan and Rosenberg, 2009; Maddison, 1997; Pamilo and Nei, 1988). Differentiation between these causes

Abbreviations: BDS, birth–death process with species sampling; bp, base pairs; CI, confidence interval; cytb, cytochrome b; GTR, general time reversible; mtDNA, mitochondrial DNA; Mya, million years ago; ND2, NADH dehydrogenase 2.

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of incongruence is essential to unravel complex speciation and diversification events.

Although phylogenetic incongruence compromises the estimation of species trees, it can be a virtue for the evolutionary biologist, who attempts to better understand complex speciation events that are often obscured by rapid diversification and hybridization (Degnan and Rosenberg, 2009; Toews and Brelsford, 2012). Genomes are mosaics of many different gene histories (Ellegren et al., 2012; Maddison, 1997; Payseur, 2010) that can be compared to deduce the species tree, while also gaining more insights into intricate events of the speciation process, such as hybridization episodes or bursts of rapid diversification (Innan and Watanabe, 2006). In addition, conflicting gene histories can also be used to infer population sizes and divergence times (Rannala and Yang, 2003; Wall, 2003). Analysing phylogenetic incongruence from a genomic perspective of diverse gene histories will lead to a more complete picture of the speciation process.

The occurrence of phylogenetic incongruence is a common and widespread phenomenon: analyses of different genes often lead to discordant gene trees (Rokas et al., 2003). Consequently, several methods have been developed to estimate the species tree from a collection of contrasting gene trees (Degnan and Rosenberg, 2009; Delsuc et al., 2005). In concatenation (or supermatrix) methods all sampled genes are concatenated and analysed as a single “supergene” (de Queiroz and Gatesy, 2007). Supertree methods, on the other hand, involve separate analyses of the sampled genes and subsequent integration of the resulting trees into a species tree (Bryant, 2003). Certain supertree methods incorporate the multispecies coalescent model to estimate the species tree from a set of heterogeneous gene trees (Knowles, 2009). The multispecies coalescent model extends the classical coalescent (Kingman, 1980) to multiple populations and describes gene trees as independent random variables generated from the coalescence process occurring along lineages of the species tree (Liu et al., 2015). A number of coalescent based methods have been developed to estimate the species tree from multigene sequence data, including Bayesian (e.g., BUCKY, Ane et al., 2007; BEAST, Drummond et al., 2012) and likelihood approaches (e.g., STEM, Kubatko et al., 2009; STELLS, Wu, 2012). Coalescent based methods have been successfully implemented to disentangle the complex evolutionary history of several closely related bird species (Carling et al., 2010; Hung et al., 2012; Lavretsky et al., 2014).

The failure to unravel the phylogenetic relationships between goose species of the tribe Anserini (commonly referred to as True Geese, see Table 1 for a taxonomic overview of this tribe) can be attributed to high levels of phylogenetic incongruence (Ruokonen et al., 2000). Although there have been several studies delving into the phylogeography of particular goose species (Humphries et al., 2009; Jonker et al., 2013; Paxinos et al., 2002; Quinn, 1992; Ruokonen et al., 2005, 2008; Scribner et al., 2003; Volkovsky et al., 2013), the phylogenetic relationships between most species remained unclear. Until now, species level phylogenies were based either on morphology (Livezey, 1996), or mitochondrial markers (Donne Gousse et al., 2002; Gonzalez et al., 2009; Lee et al., 2008; Ruokonen et al., 2000). These approaches resulted in contradicting topologies and different mitochondrial markers led to different phylogenetic reconstructions (Fig. 1). Phylogenetic incongruence is particularly apparent in the genus *Anser*. Although phylogenetic incongruence can be caused by hybridization and/or incomplete lineage sorting, all previous studies on goose phylogeny were based on mitochondrial DNA (mtDNA), which suggests analytical issues as the most likely cause of these incongruences. One example of the discrepancies among previous studies involves the phylogenetic position of the Lesser White-fronted Goose (*Anser erythropus*). Morphological analyses (Livezey, 1996) and some mitochondrial markers (cytb and ND2,

Table 1

Current taxonomy for the True Geese (tribe Anserini) based on Clements et al. (2015).

English name	Scientific name	Subspecies
<i>Genus ANSER</i>		
Swan Goose	<i>Anser cygnoides</i> ^a	
Taiga Bean Goose	<i>Anser fabalis</i>	<i>A. f. fabalis</i> ^a <i>A. f. johanseni</i> <i>A. f. middendorffii</i> <i>A. serrirostris rossicus</i> ^a <i>A. serrirostris serrirostris</i>
Tundra Bean Goose	<i>Anser serrirostris</i>	
Pink-footed Goose	<i>Anser brachyrhynchus</i> ^a	
Greater White-fronted Goose	<i>Anser albifrons</i>	<i>A. a. albifrons</i> (Eurasian) ^a <i>A. a. flavirostris</i> (Greenland) <i>A. a. gambeli</i> (Western) <i>A. a. frontalis</i> (Western) <i>A. a. elgasi</i> (Tule)
Lesser White-fronted Goose	<i>Anser erythropus</i> ^a	
Greylag Goose	<i>Anser anser</i>	<i>A. a. anser</i> (European) ^a <i>A. a. rubrirostris</i> (Siberian)
Bar-headed Goose	<i>Anser indicus</i> ^a	
Emperor Goose	<i>Anser canagicus</i> ^a	
Snow Goose	<i>Anser caerulescens</i>	<i>A. c. caerulescens</i> ^a <i>A. c. atlantica</i>
Ross' Goose	<i>Anser rossii</i> ^a	
<i>Genus BRANTA</i>		
Brent Goose	<i>Branta bernicla</i>	<i>B. b. bernicla</i> (Dark-bellied) ^a <i>B. b. hrota</i> (Pale-bellied or Atlantic) ^a <i>B. b. nigricans</i> (Black) ^a <i>B. b. orientalis</i>
Barnacle Goose	<i>Branta leucopsis</i> ^a	
Cackling Goose	<i>Branta hutchinsii</i>	<i>B. h. leucopareia</i> (Aleutian) <i>B. h. hutchinsii</i> (Richardson's) <i>B. h. minima</i> (Minima) ^a <i>B. h. taverni</i> (Taverner's)
Canada Goose	<i>Branta canadensis</i>	<i>B. c. moffitti</i> <i>B. c. maxima</i> <i>B. c. occidentalis</i> <i>B. c. fulva</i> <i>B. c. canadensis</i> ^a <i>B. c. interior</i> <i>B. c. parvipes</i>
Hawaiian Goose	<i>Branta sandvicensis</i> ^a	
Red-breasted Goose	<i>Branta ruficollis</i> ^a	

^a Blood samples retrieved from this (sub)species.

Donne Gousse et al., 2002; Gonzalez et al., 2009) report a sister relationship with the Greater White-fronted Goose (*Anser albifrons*), whereas studies using the mitochondrial control region (Lee et al., 2008; Ruokonen et al., 2000) place the Lesser White-fronted Goose in a clade with Taiga Bean Goose (*Anser fabalis*) and Pink-footed Goose (*Anser brachyrhynchus*).

In accordance with the lack of a well-supported phylogeny, the timing of origin of the extant goose species is unknown too. Fossil evidence indicates that geese were present during the Miocene and Pliocene (Brodtkorb, 1964) and several phylogeographic studies reported Pleistocene origins of certain goose subspecies (Avise et al., 1992; Shields, 1990; Van Wagner and Baker, 1990). More over, a mtDNA study of the genus *Anser* dated speciation events to the late Pliocene and early Pleistocene (Ruokonen et al., 2000). Based on the available evidence, we hypothesize that the diversification of modern goose species was initiated in the Pliocene, accompanied by further diversification into distinct subspecies during the Pleistocene. Recent developments in divergence timing methods, that integrate both genomic data and fossil calibration points, can be applied to test this hypothesis (Parham et al., 2012).

The objectives of this study were threefold: (1) to unravel the phylogenetic relationships within the Anserini tribe using phylogenomic tools for species tree estimation, (2) to assess the timing of

Fig. 1. Phylogenetic trees for the True Geese based on morphology or mitochondrial markers. (A) Morphology (Livezey, 1996). (B) Mitochondrial control region (Lee et al., 2008). (C) Cytochrome b and NADH hydrogenase 2 (Gonzalez et al., 2009).

divergence for the extant goose species by means of genomic and fossil data and (3) discuss these findings in a framework of ecological, biogeographic and climatic events.

2. Materials and methods

2.1. Sample collection and DNA extraction

We collected blood samples from 19 goose (sub)species (Table 1, see Table S1 for details). Genomic DNA was isolated from the blood samples using the Qiagen Gentra kit (Qiagen Inc.). Quality and quantity of the DNA was measured using the Qubit (Invitrogen, Life Technologies). Sequence libraries were made according to Illumina protocols and sequenced paired end (100 bp) on the Hiseq2500 (Illumina Inc.).

2.2. Processing sequences

Paired end reads (100 bp) were mapped to the Mallard (*Anas platyrhynchos*) genome, version 73 (Huang et al., 2013, supplementary material) using SMALT (<http://www.sanger.ac.uk/resources/software/smalt/>). Previous research indicated that SMALT is appropriate for mapping paired end reads when the reference genome is distantly related to the sampled species (Frantz et al., 2013). Furthermore, preliminary analyses showed that this software produces the best results compared to other mapping software (Fig. S2). SMALT uses a hash index of short words, sampled at equidistant steps along the reference genome. We applied default settings (word length = 13, spacing of hashed words = 6). Over 99% of the reads were mapped successfully in all samples, but to decrease the incidence of off site mapping we only accepted properly mapped paired reads, resulting in mapping rates between 63% and 78% (Table S2). From the resulting bam files duplicate sequences were excluded using SAMtools dedup and realigned with IndelRealigner in GATK 2.6. Variant sites were called by means of the UnifiedGenotyper in GATK 2.6 with a minimum base quality of 20 and a heterozygosity value of 0.01 (this value has proven to be suitable for variant calling after mapping to a distantly related reference genome, see Frantz, 2015). The genomic positions of exonic sequences that were one to one orthologous between the Mallard and other bird species (chicken, turkey, flycatcher and zebra finch) were retrieved from the ENSEMBL data base. These genomic positions were used to extract the sequences from the different goose genomes. A final set of 41,736 unique exons

(representing 5887 genes) were concatenated, resulting in an alignment of 6,630,626 base pairs (bp).

Paired end reads (100 bp) were also mapped to the Swan Goose (*Anser cygnoides*) genome (Lu et al., 2015) using BWA (Li and Durbin, 2009). Over 95% of the reads were mapped successfully for all species. However, mapping to the genome of a species that is included in the phylogenetic analysis (in this case the Swan Goose) potentially leads to an inherent bias that could interfere with the phylogenetic analysis. For instance, mean mapping quality (Fig. S2) is higher for species belonging to the same genus (*Anser*) as the reference genome compared to species of the other genus (*Branta*). Therefore we decided to use the variant calls based on the mapped reads to a more distantly related reference genome, the Mallard (*A. platyrhynchos*). Although this choice leads to a reduction in the amount of data, it avoids the bias introduced by mapping to the Swan Goose genome, because all species are equally distantly related to the Mallard. A similar approach has been used to reconstruct the phylogeny of Cichlid fish (Ilves and Lopez Fernandez, 2014).

2.3. Phylogenomic analyses

We applied several phylogenomic methods based on maximum likelihood implemented in RAxML 8.3 (Stamatakis, 2006). We used both supertree and supermatrix techniques (Delsuc et al., 2005). First, the concatenated alignment was analysed under two commonly used substitution models (GTR + Γ and GTR + Γ + I), both with 100 bootstrap replications (Ranwez et al., 2007). Second, we selected 3570 one to one orthologous genes (thus combining the gene exons) with a minimum length of 500 bp. These genes were analysed separately under a GTR + Γ substitution model with 100 rapid bootstraps. The resulting gene trees were filtered on average bootstrap support (minimum >50). This final set of 3558 well supported gene trees was consequently combined into a consensus tree using the software package STELLS version 1.6.1 (Wu, 2012). STELLS infers the species tree from a given set of gene trees, using the multispecies coalescent as underlying genealogical process. To assess support for particular clades in the supertree analysis, concordance factors were calculated in DensiTree (Bouckaert, 2010). A concordance factor represents the percentage of gene trees that contain the same node as the species tree (Baum, 2007; Knowles and Kubatko, 2010). Trees were rooted either by using an outgroup (Mallard), or by midpoint rooting (without an outgroup). Both methods resulted in the same position of the root in the tree. Here, we present the results of the midpoint rooting

only because the high divergence of the root species (Mallard) resulted in conflicting parameter estimations in RAxML 8.3.

2.4. Molecular clock analyses

Ideally, a molecular clock analysis is run with multiple calibration points (fossils or biogeographic events) using a relaxed clock (Parham et al., 2012). Unfortunately, the fossil record for geese is too sparse to use multiple calibration points (see Section 4). Therefore, we followed the method outlined below. The resulting divergence times should thus not be interpreted as the exact dates of divergence, but rather as rough guiding estimates that allow us to formulate testable hypotheses on the biogeographical and ecological drivers of goose speciation.

We estimated divergence times using an approximate likelihood method as implemented in MCMCtree (in PAML version 4, Yang, 2007), with a global clock and birth–death sampling. We fitted a GTR + Γ model to the concatenated alignment and estimated a mean substitution rate by fitting a strict clock and setting the root age at 9 Mya based on previous studies (Fulton et al., 2012; Jetz et al., 2012). This mean substitution rate (0.134992 ± 0.000307 substitutions/site/Myr) was used to adjust the prior on the substitution rate (rgene) modelled by a Γ distribution as $\Gamma(1, 7.4)$. Parameters for the birth–death process with species sampling (BDS) and σ^2 values were set at 110 and $G(1, 10)$, respectively. The *Anser*–*Branta* divergence was constrained between 20 and 4 million years ago to reflect reports of goose fossils in this time period (Brodkorb, 1964). MCMCtree analyses were run multiple times to check for convergence of results. Analyses with a relaxed clock gave similar results compared to the global clock approach, but without convergence of results. Therefore, we only present the estimates of the global clock analysis here. To further assess the reliability of the MCMCtree analysis, we performed a global clock calculation of divergence times using the software package PATHd8 (Britton et al., 2007), setting the root age at 9.5 Mya as a result of the MCMCtree analysis.

3. Results

The concatenation and consensus methods resulted in identical topologies (Fig. 2). All clades in the concatenation analysis were supported by maximum bootstrap values, except for the sister species relation between Tundra Bean Goose and Pink footed Goose which has a bootstrap support of 95. Analyses with the GTR + Γ substitution model and the GTR + Γ + I substitution model resulted in the same topology. There is a clear separation between the two genera (*Anser* and *Branta*). The genus *Branta* consists of a clade of White cheeked Geese – Canada Goose (*Branta canadensis*), Cackling Goose (*Branta hutchinsii*), Barnacle Goose (*Branta leucopsis*) and Hawaiian Goose (*Branta sandvicensis*) – and two basal splits leading to Brent Goose (*Branta bernicla*) and Red breasted Goose (*Branta ruficollis*). The most basal split in the genus *Anser* leads to the Bar headed Goose (*Anser indicus*). Next, two main clades can be recognised: the White Geese – Snow Goose (*Anser caerulescens*), Ross' Goose (*Anser rossii*) and Emperor Goose (*Anser canagicus*) and the Grey Geese – Greylag Goose (*Anser anser*), Swan Goose (*A. cygnoides*), the White fronted Geese (*A. albifrons* and *A. erythropus*) and the Bean Goose complex (*A. fabalis*, *Anser serrirostris* and *A. brachyrhynchus*). Concordance factors ranged between 4% and 93% (Fig. 2), with generally higher values found within *Branta* (30–93%) than within *Anser* (4–54%). Indeed, concordance factors in the consensus analysis were consistently higher for the *Branta* compared to the *Anser* (Mann–Whitney $U = 60.5$, $p = 0.015$), which indicates higher levels of phylogenetic incongruence in the latter clade.

Fig. 3 gives an overview of the divergence times (with 95% CI) for the different speciation events. The split between *Anser* and *Branta* was estimated at 9.5 Mya (15.1–4.2). This estimate was used for root calibration in the PATHd8 analysis, which resulted in similar divergence times compared to the MCMCtree analysis ($R^2 = 0.973$, $p < 0.001$, Fig. S3). Most mean divergence times are concentrated in between 4 and 2 Mya. The mean divergence times for Red breasted Goose (*B. ruficollis*) and Brent Goose (*B. bernicla*) are dated to approximately 6.8 and 5.8 Mya, respectively. Finally, three Brent Goose subspecies (*bernicla*, *hrota* and *nigricans*) originated during the Pleistocene, around one Mya. An overview of the evolutionary history of True Geese is presented in Fig. 4, combining phylogenetic relationships, divergence times and current distributions.

4. Discussion

Whole genome analyses have recently been used to unravel the phylogenetic relations between the major bird orders (Jarvis et al., 2014; Prum et al., 2015), but our study presents one of the first phylogenomic analyses of a group of closely related bird species (DaCosta and Sorenson, 2016; Nater et al., 2015). As both concatenation and consensus methods resulted in the same topology and all nodes on the concatenation tree are supported by high bootstrap values, it is likely that the resulting phylogeny closely approaches the actual species tree for the Anserini. We firmly resolve several incongruences among previous studies (see Section 4.1).

The resolution of these incongruences can be attributed to the deliberately exon based phylogenomic approach in our study (Ilves and Lopez Fernandez, 2014). First, the choice of one to one orthologous exonic sequences ensures high mapping quality because of greater sequence conservation of exons compared to introns (Kerr et al., 2014; Ranwez et al., 2007). Moreover, the use of one to one orthologous sequences avoids the analytical complexities introduced by duplicated genes (Maddison, 1997) and the occurrence of indels and greater sequence divergence that can pose substantial problems during multiple sequence alignment (Liu et al., 2010). Second, the genome wide set of thousands of genes enabled us to deduce with high statistical power the species tree despite potentially high levels of phylogenetic incongruence caused by incomplete lineage sorting and/or hybridization. Indeed, both the concatenation and the consensus method uncovered the same phylogenetic signal from the genome wide sequence data. Furthermore, the concordance factors calculated in the coalescent based consensus method provide important insights into the effects of incomplete lineage sorting and/or hybridization in certain clades.

4.1. Phylogenetic relationships

Monophyly of the genera *Anser* and *Branta* was already well established (Delacour and Mayr, 1945). However, the timing of separation of these genera is still a matter of debate. Fossil evidence suggested that these two groups of geese have a common ancestor between 5 and 4 Mya (Brodkorb, 1964; Wetmore, 1956), and this date has been used to calibrate the molecular clock in birds (Shields and Wilson, 1987). The consistency of this molecular clock has been questioned (van Tuinen and Hedges, 2001; Weir and Schluter, 2008), because subsequent molecular studies reported older dates for the *Anser*–*Branta* split ranging from 23 to 9 million years ago (Brown et al., 2008; Fulton et al., 2012; Gonzalez et al., 2009; Jetz et al., 2012; Jiang et al., 2010; Pereira and Baker, 2006). We decided to use 9 million years to calibrate the mutation rate, based on recent estimates (Fulton et al., 2012;

Fig. 2. Maximum Likelihood Tree for the True Geese based on consensus and concatenation methods. Bootstrap values of the concatenation analysis above branches, concordance factors of the consensus analysis below.

Fig. 3. Timed phylogenetic tree using a global clock approach in MCMCtree. Blue bars indicate 95% confidence intervals.

Fig. 4. An overview of the evolutionary history of the True Geese combining phylogenetic tree, divergence times and current distributions of all species. Distributions based on BirdLife's species range maps. Drawings used with permission of the Handbook of Birds of the World.

Jetz et al., 2012). Moreover, we used a fossil constraint between 20 and 4 million years ago to reflect reports of goose fossils in this time period (Brodkorb, 1964). The resulting wide confidence interval for the *Anser*–*Branta* split in our molecular clock analysis, ranging from 15.1 to 4.2 Mya, indicates that there is still considerable

uncertainty for this estimate. This uncertainty is a consequence of the lack of proper fossil calibration points for the Anserini tribe. Although there are numerous goose fossils (Brodkorb, 1964), it is not possible to confidently determine the phylogenetic position of these fossils. A thorough phylogenetic analysis of goose fossils,

Fig. 4 (continued)

such as the one recently published for the Charadriiformes (Smith, 2015), is clearly essential in order to achieve better estimates for the divergence times.

Phylogenetic relationships within the genus *Branta* (commonly referred to as the Black Geese) could be unambiguously inferred,

consisting of a clade of White cheeked Geese – Canada Goose (*B. canadensis*), Cackling Goose (*B. hutchinsii*), Barnacle Goose (*B. leucopsis*) and Hawaiian Goose (*B. sandvicensis*) – and two basal splits leading to Brent Goose (*B. bernicla*) and Red breasted Goose (*B. ruficollis*). The exact phylogenetic position of the latter two species

remained uncertain up to our study: some studies reported a sister species relation (Lee et al., 2008; Paxinos et al., 2002), while others placed either Red breasted Goose (Donne Gousse et al., 2002; Livezey, 1996) or Brent Goose at the base of the *Branta* clade (Gonzalez et al., 2009). We find strong support for a basal split leading to the Brent Goose in *Branta*. Within the White cheeked Geese clade there is a clear distinction between Canada Goose and Cackling Goose which were considered large and small bodied forms of the same species in the past (Owen, 1980). The significant morphological and molecular divergence between these species reported in previous studies is therefore confirmed by our genomic analysis (Scribner et al., 2003; Van Wagner and Baker, 1990). In addition, we find support for the sister species relation between Cackling Goose and Barnacle Goose described previously (Paxinos et al., 2002).

The Hawaiian Goose was the first species to split from the White cheeked Geese clade, around 3.5 Mya (5.5–1.5). This date suggests that the ancestors of the Hawaiian Goose arrived on islands that were present in this time period, such as Kauai, Nihoa or Oahu, (Macdonald et al., 1983). However, it is also possible that the divergence of the Hawaiian Goose occurred on the mainland, followed by colonization of Hawaii at a later stage. Our results contrast with the findings of Paxinos et al. (2002), who reconstructed the evolutionary history of the Hawaiian goose radiation (i.e. the modern Hawaiian Goose and several extinct species) using mtDNA extracted from fossil remains. They found that the Hawaiian radiation is a sister group to the Canada Goose that diverged approximately 890,000 years ago (1.3 Mya–577,000), a date also reported by Quinn et al. (1991) based on restriction fragments of mtDNA. The incongruence between our analysis and those of Paxinos et al. (2002) and Quinn et al. (1991) is probably the result of incomplete lineage sorting. A closer look at the results of the consensus method (which takes into account incomplete lineage sorting) supports this explanation. The clade comprising Canada Goose, Cackling Goose and Barnacle Goose is recovered in 35.5% of the gene trees, whereas a sister relation between Canada Goose and Hawaiian Goose is supported by 16% of the gene trees. Gene tree incongruence can also be the result of hybridization (Maddison, 1997), but this is less likely as the Hawaiian Goose has been geographically isolated from the Canada Goose. However, we cannot rule out the possibility that vagrant Canada Geese might have reached the Hawaiian archipelago in the past (Berger, 1972) or that Canada Geese and Hawaiian Geese have hybridized before the colonization of Hawaii. Another incongruence between our results and those of Paxinos et al. (2002) and Quinn et al. (1991) concerns the timing of divergence of the Hawaiian Goose (3.5 Mya versus 890,000 years ago). This large difference can be explained by the fossil calibration used by these studies as they both calibrated the molecular clock by setting the date of the *Anser*–*Branta* split at 4.5 Mya, which is later than the date we use here (9.5 Mya). In addition, the effective population size of mtDNA is smaller compared to nuclear DNA, which results in a considerably younger divergence time (Ballard and Whitlock, 2004). Therefore the Hawaiian Goose most likely diverged earlier than expected, before the further diversification of the white cheeked goose ancestor that gave rise to the Canada Goose, Cackling Goose and Barnacle Goose.

In contrast to *Branta*, the phylogenetic relationships within the genus *Anser* were largely unresolved up to this study. The failure to reconstruct the evolutionary history of this bird group can be attributed to rapid speciation, which results in incomplete lineage sorting, and hybridization (Ruokonen et al., 2000). Indeed, concordance factors in the consensus analysis were lower for the *Anser* clade compared to the *Branta* clade, indicating higher levels of gene tree discordance in the former clade. By using genomic data and methods that take into account gene tree discordance, we

were able to construct a well supported phylogeny for the genus *Anser*. The most basal split leads to the morphologically divergent Bar headed Goose (*A. indicus*). Next, two main clades can be recognised: the White Geese–Snow Goose (*A. caerulescens*), Ross' Goose (*A. rossii*) and Emperor Goose (*A. canagicus*) that are sometimes placed in a separate genus, *Chen* (Gil and Donsker, 2013), and the Grey Geese–Greylag Goose (*A. anser*), Swan Goose (*A. cygnoides*), the White fronted Geese (*A. albifrons* and *A. erythropus*) and the Bean Goose complex (*A. fabalis*, *A. serrirostris* and *A. brachyrhynchus*).

The monophyly of the White Geese was already well established from a morphological perspective (Livezey, 1996), but molecular analyses were unable to recover this clade (Donne Gousse et al., 2002; Gonzalez et al., 2009; Lee et al., 2008; Paxinos et al., 2002). We were able to confirm the monophyly of this clade, although gene tree discordance was apparent in the consensus method as only 17.1% of the gene trees supported this clade. From a taxonomic point of view the White Geese should be classified in the genus *Anser*, because placing them in the distinct genus *Chen* results in polyphyly of the genus *Anser*.

Within the Grey Geese clade, the position of the Swan Goose, as a sister species to the White fronted Geese and the Bean Goose complex, is quite surprising. Given its morphological disparity, the Swan Goose was traditionally placed at the base in the Grey Geese clade (Livezey, 1996) and has even been classified in a separate genus, *Cygnopsis* (Owen, 1980). Also, the phylogenetic position of the Lesser White fronted Goose has been established as a sister species of the Greater White fronted Goose. The placement of the Lesser White fronted Goose in a clade with Taiga Bean Goose and Pink footed Goose by previous studies can be explained by analytical shortcomings, incomplete lineage sorting and/or hybridization (Lee et al., 2008; Ruokonen et al., 2000).

The taxonomy and evolutionary relationships of the Bean Goose complex have been controversial (Delacour, 1951; Mooij and Zöckler, 1999; Sangster and Oreel, 1996). The current classification encompasses three species: the Pink footed Goose, the Taiga Bean Goose (three subspecies) and the Tundra Bean Goose (two subspecies). However, based on analyses of the mitochondrial control region, Ruokonen et al. (2008) identified three distinct lineages: the Pink footed Goose, the Middendorff's Goose (currently a subspecies of Taiga Bean Goose), and the Bean Goose (currently split in Taiga and Tundra Bean Goose). We report a sister species relation between Pink footed Goose and Tundra Bean Goose. The phylogenetic relationships in this complex are highly influenced by incomplete lineage sorting and/or hybridization. A thorough genomic analysis in a phylogeographic context with broad sampling across the entire range of this species group is needed to confidently reconstruct the evolutionary history of the Bean Goose complex.

4.2. Biogeography and ecology

It can be conceived that True Geese originated in Eurasia, considering the fact that the earliest (Miocene) goose fossils have been excavated in Western Europe (Brodkorb, 1964) and species at the phylogenetically most basal splits (Bar headed Goose, Brent Goose and Red breasted Goose) currently have a largely Eurasian distribution (although the current distribution does not necessarily coincide with the past distribution). If we assume a Eurasian origin, at least three colonization events to North America can be recognised: by the White cheeked Geese, by the White Geese, and finally by the Greater White fronted Geese. However, this scenario remains to be tested by means of ancestral reconstructions in combination with fossil data. Based on the phylogeny and present day biogeographic patterns, we can recognize three main groups: the Black Geese (*Branta*) with members in North America and Eurasia,

the Grey Geese (*Anser*) with a largely Eurasian distribution, and the North American White Geese (*Anser*).

Within these three groups, there is substantial variation in beak morphology which is most pronounced in the Grey Geese of the genus *Anser*. The Grey Geese can be regarded as an adaptive radiation, because they show “the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage” (Schluter, 2000). To qualify as an adaptive radiation, four criteria should be met: (1) common ancestry, (2) rapid speciation, (3) phenotype environment correlation, and (4) trait utility (Schluter, 2000). The first two criteria (common ancestry and rapid speciation) are supported by our phylogenomic analyses. All members of the Grey Geese share a common ancestor around 3.4 Mya and diversified into several distinct species over a period of about one million years. A phenotype environment correlation indicates that differences in phenotypes are associated with the use of different resources or environments. For geese, this correlation has been confirmed by numerous field studies (Carriere et al., 1999; Fox and Bergersen, 2005; Giroux and Bergeron, 1996). For instance, the Taiga Bean Goose has a long thin bill adapted for probing soft substrates of bogs and marshlands to feed on underground plant material, whereas the Pink footed Goose has a short bill suitable for grazing and seed stripping (Owen, 1976; Reed, 1976). Finally, the trait utility (i.e., evidence that morphological or physiological traits are indeed useful when used in the associated environment) of beak morphology in geese has been confirmed experimentally (Durant et al., 2003; Heuermann et al., 2011; Zhang et al., 2015). The Grey Geese can thus be regarded as an adaptive radiation in terms of beak morphology.

The diversification in beak morphology is less pronounced in the Black Geese and White Geese, which can be attributed to the fact that some members of the Black Geese (specifically, the White cheeked Geese) and the White Geese coexisted in North America and consequently affected each other in terms of beak morphology evolution by means of resource competition and consequent character displacement (Dayan and Simberloff, 2005; Schluter, 2000). So, while the Eurasian niches were rapidly filled by members of one ancestral Grey Goose lineage, the North American niches were filled by members of two distantly related lineages: the Black Geese and the White Geese. One can see every North American species as the ecological equivalent of a Eurasian species (or vice versa), for example, Owen (1980) remarked that “the larger races of the Bean Goose [are] the Canada’s [goose] ecological counterpart in Eurasia.” However, it is also possible that the Black Geese and White Geese both exhibited an adaptive radiation in North America comparable with the Eurasian radiation of the Grey Geese, but that several species went extinct during the Pleistocene (Martin and Klein, 1989).

We hypothesized a Pliocene origin for modern goose species followed by diversification at the subspecies level during the Pleistocene, based on the available fossil evidence (Brodkorb, 1964) and previous molecular studies (Avice et al., 1992; Ruokonen et al., 2000; Scribner et al., 2003; Van Wagner and Baker, 1990). Indeed, our analysis indicates that the majority of speciation events took place at the end of the Pliocene. The approximate date of diversification coincides with the beginning of a period of climatic oscillations between 3.2 and 1.9 million years ago. This period was part of a fast global cooling trend, following the closure of the Panama Seaway and the uplifting of the Tibetan Plateau around four million years ago (Zachos et al., 2001). This resulted in the formation of permanent Northern Hemisphere ice sheets, the establishment of a circumpolar tundra belt and the emergence of temperate grasslands, which opened up new ecological niches in which new groups of animals and plants were able to spread (Kahlke, 2014; Prins, 1998). The tundra habitat serves as breeding ground for geese (Owen, 1980), while the temperate grasslands act as winter

ing grounds where mate choice takes place (Rodway, 2007). Moreover, these tundra and grassland habitats provided ample opportunity for geese to explore new ecological niches and diversify in beak morphology.

5. Conclusions

Using a phylogenomic approach we were able to resolve the contentious phylogenetic history of the True Geese. Furthermore, taking advantage of the many contrasting gene histories, we gained more insight into the effects of complex speciation processes, such as rapid diversification and hybridization, in certain clades. The relative importance of hybridization in the evolutionary history of the True Geese remains to be investigated. The wide spread occurrence of hybridization in birds (Ottenburghs et al., 2015), and specifically waterfowl (Kraus et al., 2012; Randler, 2008; Ottenburghs et al., 2016), suggests that hybridization can act as an important component in avian speciation (Rheindt and Edwards, 2011). By integrating over the full exon set of genes we made a first step to quantitatively describe both species and gene histories. Our approach will be a fruitful strategy for resolving many other complex evolutionary histories at the level of genera, species, and subspecies.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2016.05.021>.

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