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Population genomics reveals speciation processes in the Mallard lineage

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Abstract. The Mallard (*Anas platyrhynchos* Linnaeus, 1758) is a well-known dabbling duck, the most numerous and widely distributed in the world. Despite being a thoroughly studied species in all aspects of its biology, ecology, behavior, and genetics, the Mallard population structure in the Holarctic remains a complicated issue. We used 2,529 polymorphic loci obtained via double-digest restriction-site associated DNA sequencing (ddRAD-seq) to reexamine the genetic differentiation between populations of the Mallard and one of its close relatives in Eastern Asia, the Eastern Spot-billed Duck (*Anas zonorhyncha* Swinhoe, 1866). Comprehensive sampling allowed us to select only pure wild mallards for our research, resulting in a final dataset of ddRAD-seq profiles from 821 mallards and 23 Eastern spot-billed ducks. Population structure analysis confirmed weak genetic structure between wild mallards from Eurasia and North America attributable to small frequency differences across autosomal loci as well as the strong structuring of Greenland mallards. The differentiation of the Greenland Mallard (*A. p. conboschas*) from the nominate subspecies (*A. p. platyrhynchos*) was higher than the differentiation between the Mallard (*A. p. platyrhynchos*) and the Eastern Spot-billed Duck (*A. zonorhyncha*) based on both autosomal and Z-linked loci. The Eastern Spot-billed Duck was found to be more closely related to mallards from Eurasia than to mallards from North America at autosomal loci but differed from both groups at Z-linked loci. Our findings are compatible with three evolutionary scenarios: (i) incipient neutral divergence between Eurasian and North American mallards, (ii) divergence-with-gene-flow potentially supported by sex-linked barriers between Mallard and Eastern Spot-billed Duck, and (iii) divergence associated with founder effects and local adaptation in the Greenland Mallard.

Key words: Mallard; Eastern Spot-billed Duck; population genomics; population structure; speciation

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Data availability statement. Data are available from FigShare: doi 10.6084/m9.figshare.31884205

Популяционная геномика раскрывает процессы видообразования в линии кряквы

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Аннотация. Кряква (*Anas platyrhynchos* Linnaeus, 1758) – один из самых известных и широко распространенных видов пластинчатоклювых птиц. Несмотря на то что этот вид тщательно изучен во всех аспектах его биологии, экологии, поведения и генетики, популяционно-генетическая структура кряквы в Голарктике до сих пор вызывает много вопросов. Для изучения генетической дифференциации между популяциями кряквы и близкородственным ей видом в Восточной Азии пестроносой кряквой (*Anas zonorhyncha* Swinhoe, 1866) были использованы 2529 полиморфных ddRAD-локусов. Для исследования были отобраны только «чистые» дикие кряквы (без примеси ДНК фермерских крякв или близкородственных видов), в результате чего получен итоговый набор ddRAD-seq данных для 821 кряквы и 23 пестроносых крякв. Анализ популяционной структуры подтвердил слабую генетическую структурированность диких крякв Евразии и Северной Америки, обусловленную небольшими различиями в частотах аллелей по аутосомным локусам, а также значительную дивергенцию гренландской кряквы (*A. p. conboschas*). Генетическая дифференциация гренландской кряквы и номинативного подвида кряквы (*A. p. platyrhynchos*) оказалась выше, чем кряквы (*A. p. platyrhynchos*) и пестроносой кряквы (*A. zonorhyncha*) как по аутосомным, так и по Z-сцепленным локусам. Показано, что пестроносая кряква по аутосомным локусам генетически ближе к евразийским популяциям кряквы, чем к

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североамериканским, тогда как по Z-сцепленным локусам она отличается от обеих групп. Полученные нами данные согласуются с тремя эволюционными сценариями: 1) начальная нейтральная дивергенция между евразийскими и североамериканскими популяциями кряквы; 2) дивергенция при потоке генов, потенциально поддерживаемая сцепленными с полом изолирующими механизмами, между кряквой и пестроносой кряквой; 3) дивергенция, связанная с эффектом основателя и локальной адаптацией у гренландской кряквы.
Ключевые слова: кряква; кряква пестроносая; популяционная геномика; популяционная структура; видообразование

Introduction

The Mallard (*Anas platyrhynchos* Linnaeus, 1758) is a migratory species with a complex population structure. On the one hand, it possesses high dispersal ability with many individuals undertaking flights of over a thousand kilometers annually as part of their seasonal migrations (Kulikova et al., 2005). On the other hand, there are resident populations in Europe as well as sedentary feral populations consisting of wild and domestic mallards in urban areas throughout the world (Young, 2005). Furthermore, in Europe and North America, game-farm mallards have been released for decades to facilitate hunting and to prevent the decline of wild populations.

Such releases have had an appreciable impact on the genetic structure of wild populations due to hybridization and subsequent gene flow (Söderquist et al., 2017; Lavretsky et al., 2023b). The Mallard also hybridizes with closely related species worldwide wherever they come into contact (reviewed in Kulikova, Zhuravlev, 2009), a process that Champagnon et al. (2023) argue is exacerbated by massive releases of game-farm mallards, which increase the frequency and geographic extent of such interspecific hybridizations. Six of the 14 species from the Mallard superspecies complex reside in the Holarctic. Excluding the Mallard itself, these are the Eastern or Chinese Spot-billed Duck (*Anas zonorhyncha*) and the Indian Spot-billed Duck (*A. poecilorhyncha*) in Eurasia; the American Black Duck (*A. rubripes*), Mottled Duck (*A. fulvigula*), and Mexican Duck (*A. diazi*) in North America (Baldassarre, 2014).

The population genetic structure of the Mallard and its closely related species has gained considerable attention over the last 40 years beginning with phylogeographic studies based on RFLP (Restriction Fragment Length Polymorphism) of mitochondrial DNA (mtDNA) and sequencing of mtDNA genes (Avise et al., 1990; Rhymer et al., 1994; McCracken et al., 2001; Kulikova et al., 2005; Kraus et al., 2011); microsatellite analysis (Mank et al., 2004; Williams et al., 2005; Ford et al., 2017); studies of nuclear DNA polymorphisms by sequencing introns of nuclear genes (Lavretsky et al., 2014; Peters et al., 2014a, b), and eventually, the use of multiple single nuclear polymorphisms (SNPs) randomly scattered across genomes obtained via double-digest restriction-site-associated DNA sequencing (ddRAD-seq) (Kraus et al., 2013; Lavretsky et al., 2015, 2019a, b; Kulikova et al., 2022; Kaminski et al., 2024). Studies using different molecular genetic markers have revealed somewhat dissimilar patterns of genetic differentiation of Mallard populations in the Holarctic. Phylogeographic analyses based on variability of the mtDNA control region discovered weak population structure with a negligible portion of genetic variance between currently recognized waterfowl flyways within Eurasia and North America but high genetic differentiation between these two landmasses (Kulikova et al., 2005; Kraus et al., 2011; Lavretsky et al., 2023b). Initially, large-scale data on nuclear SNP set comprising hundreds of loci used in a population genomic study failed to detect population genetic structure in the Mallard across the Holarctic. It was suggested that "the world's mallards perhaps with minor exceptions form a single large, mainly interbreeding population" (Kraus et al., 2013).

However, recent ddRAD-seq analyses have uncovered fine-scale nuclear structure among wild mallards, identifying three primary groupings: mainland Eurasia, North America, and Greenland, with the latter being the most genetically distinct (Lavretsky et al., 2023b). Wild mallards from Eurasia and North America showed subtle but clear nuclear substructuring attributable to small frequency differences across their genomes. A series of studies on hybridization between the Mallard and its closely related species in North America showed that extensive genomic introgression is more limited than previously assumed with species boundaries maintained by a combination of behavioral mechanisms, reduced fitness of hybrids, and rapid backcrossing that restores parental genotypes within a few generations (Lavretsky et al., 2019a, b, 2023b).

This study aims to integrate ddRAD-seq data on the population genomic structure of the Mallard across the Holarctic with data on hybridization between the Mallard and the Eastern Spot-billed Duck in Northeast Asia in order to elucidate patterns of genomic divergence in the Mallard lineage. ddRAD-seq has been widely and successfully applied in dabbling duck population genomics studies (Lavretsky et al., 2015, 2019a, 2023a, b; Kaminski et al., 2024; Kulikova et al., 2022, 2025). So, to achieve our goal, we utilized ddRAD-seq data from genetically vetted wild mallards collected in regions with a low probability of hybridization with game-farm mallards and confirmed as not having been admixed with game-farm ducks (Lavretsky et al., 2023b). This careful filtering ensures that our analyses reflect natural evolutionary processes rather than anthropogenic introgression. Specifically, our main objectives were: (1) to compare patterns of genomic divergence between Mallard populations from two continents and between the Mallard and its close relative, the Eastern Spot-billed Duck; and (2) to make inferences on the speciation patterns and evolutionary history of the Mallard lineage in the Holarctic that have shaped its current genomic diversity.

Materials and methods

A total of 821 individual samples of wild mallards collected across the Mallard range in Holarctic and 23 individual samples of Eastern spot-billed ducks from the southeast of the Russian Far East representing the northern part of the Eastern



Fig. 1. Geographical locations of samples obtained across the Holarctic.
On the map, wild mallards are denoted by light-grey circles and Eastern Spot-billed Ducks are denoted by dark-grey circles.

Spot-billed Duck range (Fig. 1; Supplementary Table S1)[1] were included in our analyses.

Samples were grouped into populations based on geographic origin and major flyways. European samples included those from across Europe (including the UK, Fennoscandia, Iceland, the Faroe Islands, and continental Europe from France to European Russia). The Northeast Asian population comprised samples from the Russian Far East and China, while Siberian samples originated from the Omsk and Tomsk regions. Greenland samples were collected from western Greenland (Nuuk and Qeqqata). North American populations were assigned to four major flyways (Blohm et al., 2006: Fig. 1). The Pacific Flyway included samples from Alaska, California, and British Columbia; the Central Flyway, from the central United States and the Canadian Prairies (Saskatchewan and Alberta); the Mississippi Flyway, from the upper Midwest, the Mississippi Alluvial Valley, and Ontario (including Manitoba); and the Atlantic Flyway, from the eastern United States and Quebec. Eastern Spot-billed Ducks were represented by samples from the southern Russian Far East (Primorye). We used published ddRAD-seq data for the Mallard (BioProject PRJNA516035, Lavretsky et al., 2019b; BioProject PRJNA828171, Kulikova et al., 2022; BioProjects PRJNA980669, PRJNA800412, and PRJNA718623, Lavretsky et al., 2023b; BioProject PRJNA911832, Schummer et al., 2023) and for the Eastern Spot-billed Duck (BioProject PRJNA828171, Kulikova et al., 2022). From the dataset of Lavretsky et al. (2023b), we extracted ddRAD seq data for genetically "pure" wild mallards guided by the criterion of admixture, i. e., when the probability of sample assignment to the game-farm mallards genetic cluster was less than 10 %. Fasta files from two datasets (Kulikova et al., 2022; Lavretsky et al., 2023b) were aligned against each other with BLASTn v.2 (Altschul et al., 1990); shared ddRAD-loci were aligned with MUSCLE v.3.8.31 (Edgar, 2004) with gaps removed using fastagap (https://github.com/nylander/fastagap) and used in subsequent analyses.

For population genetic analyses, we aggregated sampling localities into biological populations (Table S1) based on hypothesized Mallard migration corridors in Europe, Asia, and North America (Boere et al., 2006). Nuclear population structure was assessed using bi-allelic ddRAD-seq SNPs. SNP-sites v.2.5.1 (Page et al., 2016) was used to extract all bi-allelic SNPs from the concatenated FASTA alignment and to export them into a VCF-like file, which was then filtered using BCFtools v.1.23 (Danecek et al., 2021) by removing singletons, SNPs missing data in ≥5 % of samples, and SNPs with a minor allele frequency (MAF) ≤ 0.02. In addition, we extracted one bi-allelic SNP per locus applying the same filters. For Z-chromosome analyses, we used only males (ZZ) as their diploid genotype allowed for direct comparison with autosomal data.

To visualize genetic structure, we used Principal Components Analysis (PCA) as implemented in the R package 'dartR' v.2.9.9.5 (Mijangos et al., 2022). Next, we examined population structure using the Bayesian clustering method implemented in STRUCTURE v.2.3.4 (Falush et al., 2003), which was run using an admixture model and correlated allele frequencies without prior information regarding species or population designation. The degree of admixture (α) was inferred from the data with a separate α parameter estimated for each population to account for potential heterogeneity in demographic histories among clusters. Ten replicates for each value of K in the range of 1–10 were run with 100,000 steps of the MCMC after a 50,000-step burn-in for each run. We applied StructureSelector (Li, Liu, 2018) to process STRUCTURE output files and to determine the optimum K based on the calculation of ΔK (Evanno et al., 2005) and MedMedK/MaxMedK estimators (Puechmaille, 2016). The latter were

[1] Supplementary Tables S1–S3 and Figure S1 are available at: https://vavilovj-icg.ru/download/pict-2026-30/appx....zip

used to account for the highly uneven sampling across the nine predefined populations (ranging from 11 to 302 individuals). We also calculated relative divergence values (Φ_{ST}) based on concatenated nucleotide sequences of all autosomal and Z-chromosome loci between the genetic clusters identified by STRUCTURE using the R package 'PopGenome' v.2.7.7 (Pfeifer et al., 2014). We then compared these values to the net nucleotide distance (d_a) estimated by STRUCTURE between the same clusters. Together these metrics provide complementary insights in which d_a shows the evolutionary distance between populations and Φ_{ST} reflects the degree of population structure resulting from genetic drift, reduced gene flow, and accumulated mutations.

We assessed pairwise population genetic differentiation by calculating standardized F_{ST} values (Meirmans, 2006) in the 'dartR' R package v.2.9.9.5. This metric provides a comparable measure of differentiation across populations with varying levels of within-population diversity. The analysis was based on 1,000 bootstrap replicates to generate 95 % confidence intervals. The resulting matrix of pairwise standardized F_{ST} values was corrected for negative eigenvalues using the Lingoes transformation (Lingoes, 1971) to ensure its Euclidean properties. Subsequently, Principal Coordinates Analysis (PCoA) was performed on the corrected distance matrix using the *dudi.pco* function in the R package 'adegenet' v.2.1.3 (Jombart, 2008).

Allelic richness (AR), mean expected heterozygosity (Nei's gene diversity), and mean observed heterozygosity within populations were estimated using the R package 'hierfstat' v.0.5-11 (Goudet, 2005). To assess the robustness of estimates and calculate confidence intervals a non-parametric bootstrap procedure with 500 iterations was performed resampling loci with replacement in each iteration. Finally, we used the 'PopGenome' v.2.7.7 to calculate composite species-pairwise and population-pairwise estimates of relative divergence (Φ_{ST}), absolute divergence (d_{XY}), and nucleotide diversity (π) for concatenated FASTA files of autosomal and Z-sex chromosome ddRAD-seq loci. In addition to composite pairwise population estimates, per-locus estimates of relative differentiation were also calculated across all ddRAD-seq loci.

Results

The aligned dataset comprised 2,387 autosomal loci (288,578 aligned nucleotides and 39,199 SNPs) and 142 Z-chromosome loci (17,809 aligned nucleotides and 1,173 SNPs), with a total of 306,387 aligned nucleotides and 40,372 SNPs.

Similar levels of autosomal nucleotide diversity were recovered across all populations (average π range = 0.0051–0.0054) except for Greenland mallards, which had lower nucleotide diversity (0.00415; Table S2). Z-chromosome nucleotide diversity across Mallard populations from Eurasia and North America was two times lower than autosomal nucleotide diversity (average π range = 0.00225–0.00245) and was further depressed in Greenland mallards and Eastern spot-billed ducks (~0.0014 and ~0.0012, respectively). The same pattern was observed for allelic richness and observed heterozygosity. Namely, the Greenland mallards showed decreased values at both autosomal and Z-linked loci whereas the Eastern spot-billed ducks exhibited values similar to those of Eurasian and North American Mallard populations at autosomal loci but a more pronounced decrease at Z-linked loci (Table S2).

Population structure

Plotting the first two principal components from the PCA of autosomal loci revealed evidence of differentiation among populations. PC1 clearly separated Greenland mallards from the other groups while PC2 differentiated Eastern spot-billed ducks, Eurasian mallards, and North American mallards, which were dispersed along PC2 in orderly, partially overlapping clusters (Fig. 2*A*). The cluster of Eastern spot-billed ducks was adjacent to mallards from Northeast Asia along

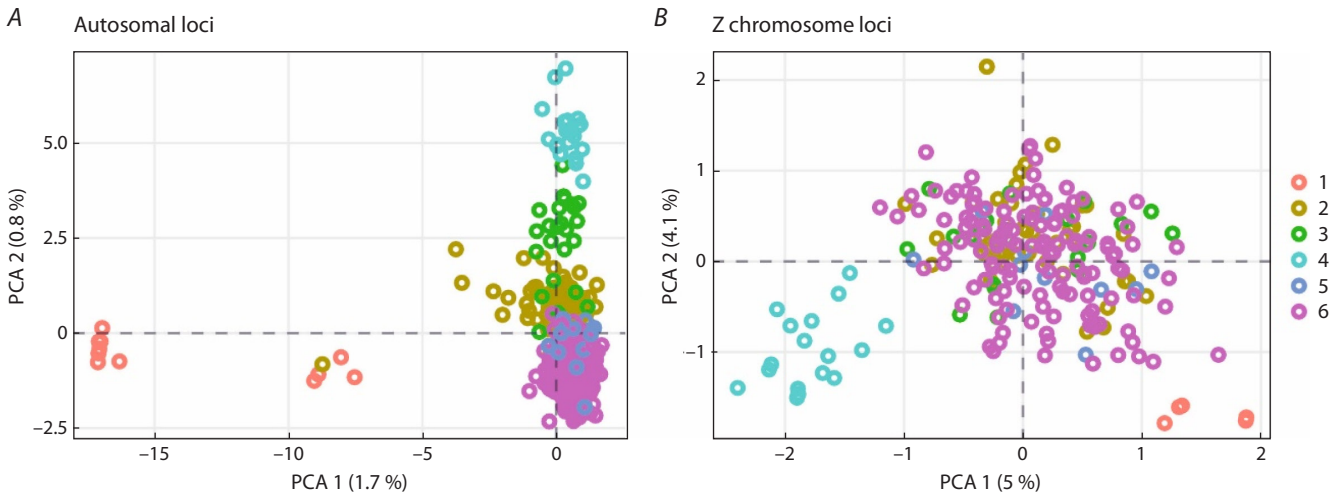


Fig. 2. Principal component analysis (PCA) based on 36,987 autosomal (*A*) and 1,135 Z-chromosome (*B*) bi-allelic SNPs assayed across 844 and 510 samples, respectively, comprising wild mallards and Eastern spot-billed ducks.

1 – Greenland Mallard; 2 – Mallard, Europe; 3 – Mallard, Northeast Asia; 4 – Eastern Spot-billed Duck; 5 – Mallard, NA Pacific Flyway; 6 – Mallard, NA Atlantic Flyway.

PC2. Greenland mallards formed two clusters differentiated by PC1 with one of them occupying an intermediate position between the other Greenland Mallard cluster and all remaining North American and Eurasian mallards. Plotting the first two principal components from the PCA of Z-chromosome loci illustrated divergence among Greenland mallards, Eastern spot-billed ducks, and all other Mallard samples from North America and Eurasia (Fig. 2*B*). The latter formed a single cluster with individuals from different populations scattered randomly.

For the STRUCTURE analysis, we used single biallelic SNPs randomly chosen from each locus resulting in a total of 2,164 biallelic autosomal SNPs and 104 Z-chromosome SNPs. This selection was made to satisfy the model's assumption of no linkage disequilibrium between markers. Prior to analysis, SNPs were filtered to exclude singletons, loci missing in ≥5 % of samples across the dataset, and those with a minor allele frequency (MAF) ≤ 2 %. We analyzed autosomal and Z-chromosome biallelic SNPs separately.

The STRUCTURE analysis for autosomal loci identified K = 3 as the optimal number of genetic clusters, strongly supported by both the Evanno method (sharp, definitive peak ΔK, Fig. 3*B*) and the consensus of the Puechmaille method (MedMedK = 3, MedMeaK = 3, MaxMeaK = 3; Fig. S1*A*). The bar plot for K = 3 was selected for downstream analysis and interpretation. It is worth noting that adding more clusters did not alter the pattern of the three main genetic groups identified by the analysis (Fig. 3*A*). The first genetic cluster identified by STRUCTURE comprised Greenland mallards, the second cluster included mallards from Eurasia and Eastern spot-billed ducks, and the third cluster consisted of mallards from all North American samples. One Greenland mallard showed evidence of admixture from North American mallards (Q = 0.449), and three Greenland mallards demonstrated admixture with both North American and Eurasian mallards with Q-values ranging from 0.179–0.240 and 0.212–0.282, respectively. One mallard from Europe (specifically from the UK) showed evidence of admixture from Greenland mallards (Q = 0.552).

The STRUCTURE analysis for Z-linked loci also revealed the highest support for K = 3 as the optimal number of genetic clusters. This inference was consistently supported by the ΔK calculation (Fig. 3*D*) and the MedMedK & MaxMedK estimators from the Puechmaille method (Fig. S1*B*). Three genetic populations assigned by STRUCTURE were composed of Greenland mallards, Eastern spot-billed ducks, and mallards from North America and Eurasia (Fig. 3*C*). Two mallards from Europe (sampled in Iceland) were admixed with Greenland mallards with Q-values of 0.566 and 0.774 as were several mallards from the Atlantic Flyway with Q-values ranging from 0.234 to 0.752 (Fig. 3*C*). A highly admixed mallard with Greenland Mallard ancestry was discovered in the Mississippi Flyway (specifically from the state of Kentucky, Q = 0.843).

We also assessed genetic differentiation between clusters identified by STRUCTURE using absolute (d_a, Nei, Li, 1979; Falush et al., 2003) and relative (Φ_{ST}, Excoffier et al., 1992) genetic divergence parameters based on SNP allele frequencies and concatenated nucleotide sequences of all loci, respectively (see the Table). The highest values of absolute and relative divergence were observed for the Greenland Mallard genetic cluster, which was the most differentiated for both autosomal and Z-linked loci. The lowest values (d_a = 0.0012 and Φ_{ST} = 0.00473) were detected between the North American Mallard cluster and the cluster comprising Eurasian mallards and Eastern spot-billed ducks based on autosomal loci. These estimates were approximately 25–29 times lower than those between these clusters and the Greenland Mallard cluster. For Z-linked loci, the divergence parameters between the most (Greenland Mallard) and least (Mallard) differentiated genetic clusters differed by a factor of 1.6 to 2.8 (see the Table). Overall, the two metrics showed a strong positive correlation for both autosomal and Z-linked markers (r = 0.999 for both), suggesting that genetic populations with greater absolute divergence also exhibit stronger relative differentiation.

Population differentiation

The PCoA of population-standardized pairwise F_{ST} values calculated from biallelic SNP datasets revealed a pattern of genetic relationships among the sampled populations broadly similar to that observed in the STRUCTURE analysis (Fig. 4). In the PCoA plot for autosomal loci, the first axis explaining 96.6 % of the variance clearly separated the Greenland mallards from the remaining populations while the second axis (2.5 % of the variance) showed some distinction between North American and Eurasian Mallard populations with the Eastern Spot-billed Duck clustering closer to Eurasian mallards, specifically to those from Northeast Asia (Fig. 4*A*).

For the Z-chromosome loci, the first two coordinates explained 41.4 and 14.4 % of the total genetic variation, respectively. The plot revealed a clear separation of Greenland mallards and Eastern spot-billed ducks from each other and from the other populations along the first axis, whereas the second axis did not distinguish between North American and Eurasian Mallard populations with the sole exception of mallards from Siberia which were set apart from the rest (Fig. 4*B*).

Pairwise genetic differentiation among populations was also visualized using a heatmap of relative divergence values (Φ_{ST}). The Φ_{ST} values for autosomal markers ranged from 0.00027 to 0.13328 (Fig. 5*A*) and for Z-chromosome markers, from 0.00022 to 0.35184 (Fig. 5*B*). The heatmap revealed the distinct separation of the Greenland Mallard with the greatest relative divergence observed between the Greenland and the other Mallard populations based on both autosomal (Φ_{ST} = 0.11661–0.12693) and Z-chromosome (Φ_{ST} = 0.22114–0.23074) loci. The Eastern Spot-billed Duck displayed significantly higher divergence from the other Mallard populations based on Z-linked loci (Φ_{ST} = 0.11747–0.17487) than on autosomal loci (Φ_{ST} = 0.01769–0.02091). The highest Φ_{ST} values were detected between the Greenland Mallard and the Eastern Spot-billed Duck: Φ_{ST} = 0.13328 for autosomal loci and Φ_{ST} = 0.35184 for Z-linked loci. Mallard populations in North America were the most genetically similar with pairwise $\Phi_{ST} < 0.00461$ for autosomal and $\Phi_{ST} < 0.0105$ for Z-chromosome markers, in contrast to Eurasian Mallard populations, which were more differentiated with pairwise $\Phi_{ST} < 0.01231$ for autosomal and $\Phi_{ST} < 0.02411$ for Z-chromosome markers.

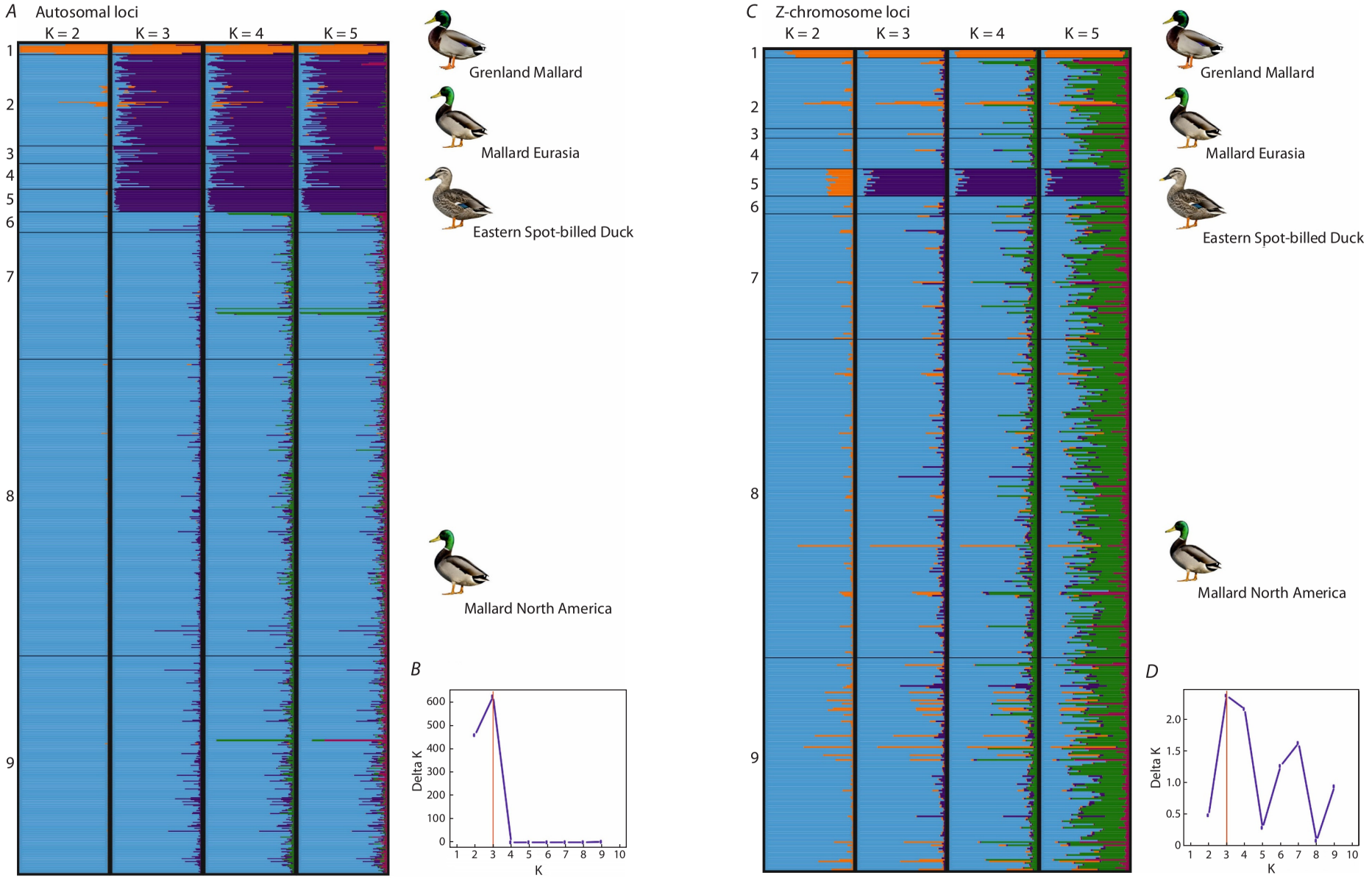


Fig. 3. *A*, STRUCTURE assignment probabilities for mallards and Eastern spot-billed ducks based on 2,164 autosomal ddRAD-seq loci, shown for K = 2 to 5. *B*, Plot of ΔK values against the number of clusters (K) (following Evanno et al., 2005) for 2,164 autosomal loci, used to determine the optimal K. *C*, STRUCTURE assignment probabilities based on 104 Z-linked ddRAD-seq loci (K = 2 to 5). *D*, Plot of ΔK values against K for the 104 Z-linked loci.

1 – Greenland Mallard; 2 – Mallard, Europe; 3 – Mallard, Siberia; 4 – Mallard, Northeast Asia; 5 – Eastern Spot-billed Duck; 6 – Mallard, NA Pacific Flyway; 7 – Mallard, NA Central Flyway; 8 – Mallard, NA Mississippi Flyway; 9 – Mallard, NA Atlantic Flyway.

Relative divergence values (Φ_{ST}, above diagonal) and net nucleotide distances (d_a, below diagonal) between genetic clusters identified by STRUCTURE for autosomal and Z-linked loci

Autosomal loci	Greenland Mallard	Eur Mall & SBDU	NA Mallard
Greenland Mallard		0.11754	0.12034
Eur Mall & SBDU	0.03532		0.00473
NA Mallard	0.03517	0.0012	
Z-linked loci	Greenland Mallard	SBDU	Mallard
Greenland Mallard		0.35184	0.2209
SBDU	0.03581		0.12587
Mallard	0.02331	0.0146	

Note. SBDU – Eastern Spot-billed Duck, NA Mallard – mallards from North America, Eur Mall & SBDU – mallards from Eurasia and Eastern spot-billed ducks.

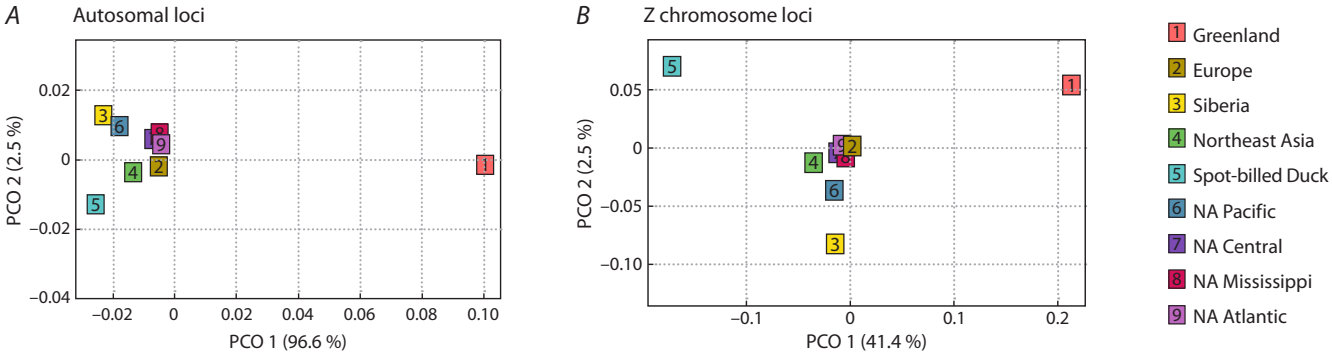


Fig. 4. Principal coordinate analysis (PCoA) based on standardized pairwise F_{ST} values (Meirmans, 2006) among eight Mallard and one Eastern Spot-billed Duck population for (*A*) autosomal loci and (*B*) Z-chromosome loci.

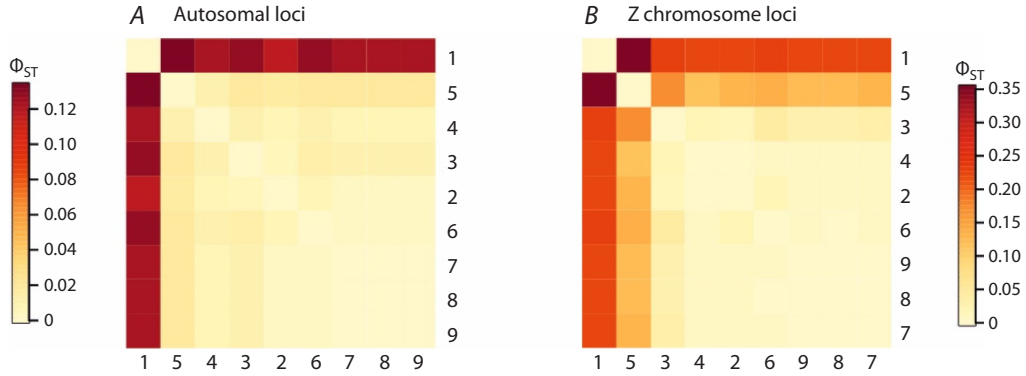


Fig. 5. Heatmap depicting relative divergence (Φ_{ST}), ranging from low (light yellow) to high (dark red), among eight Mallard and one Eastern Spot-billed Duck population for (*A*) autosomal loci and (*B*) Z-chromosome loci.

Population numbers follow those in the legend of Figure 3.

Discussion

Greenland Mallard

Among the Eastern Spot-billed Duck and eight Mallard populations studied, the Greenland Mallard was identified as the most genetically differentiated (Figs 2–5, see the Table). It also exhibited the lowest genetic diversity based on both autosomal and Z-chromosome loci (Table S2). These two facts are likely the result of a founder effect and prolonged isolation of this endemic Greenland subspecies (*Anas platyrhynchos conboschas* Brehm, CL, 1831), which breeds in western and eastern Greenland (Alerstam et al., 1986; Lyngs, 2003). Similar results regarding the genetic uniqueness of the Greenland Mallard were obtained previously in studies of Mallard population genetic structure using mtDNA, 363 nuclear SNP markers, and ddRAD-seq data (Kraus et al., 2011, 2013; Lavretsky et al., 2023b). Lavretsky et al. (2023b) attributed the Greenland Mallard's distinctiveness to a severe

population bottleneck and subsequent genetic drift resulting in the detection of 123 to 670 putative outlier loci including 66 consistent "islands of differentiation" across the genome. Furthermore, the demographic reconstructions of Lavretsky et al. (2023b) placed the Greenland Mallard among highly bottlenecked feral populations such as those in Hawaii and domestic Khaki Campbells which exhibited deviations from the ancestral wild demographic state.

What is most striking about the Greenland Mallard is its higher differentiation from the nominate subspecies (*A. p. platyrhynchos* Linnaeus, 1758) based on both autosomal and Z-linked loci (Φ_{ST} = 0.123 and 0.225, respectively) than the differentiation between the Mallard and the Eastern Spot-billed Duck (Φ_{ST} = 0.0185 and 0.135, respectively). The difference in genetic differentiation between two subspecies and two closely related species is supported by PCA and STRUCTURE analyses (Figs 2 and 3). This pattern is consistent with a scenario of peripatric speciation, in which an endemic Mallard population has diverged through adaptation to *Greenland's* high-latitude environment. The Greenland Mallard differs from the nominate Mallard subspecies in being larger, with a somewhat shorter bill, darker upperparts, and much more heavily spotted underparts (Schiøler, 1905). This description is consistent with classic biogeographic principles representing an example of both Bergmann's rule and Allen's rule in birds (Bergmann, 1847; Allen, 1877). Bergmann's rule, which posits that polar forms tend to be larger than their conspecifics from warmer climates, is evident in the greater body size of the Greenland Mallard compared to the nominate subspecies *A. p. platyrhynchos*. Allen's rule, which states that appendages are smaller in colder climates to minimize heat loss, is displayed in the Greenland Mallard's proportionally smaller bill and stockier overall build (Salomonsen, 1950). The evolutionary process of accumulation of phenotypic and genetic differences in an island Mallard population is currently evident in the distinct morphology of the subspecies and in its marked genetic divergence from the nominate form.

Interestingly, genetic heterogeneity of the Greenland Mallard was demonstrated by PCA and STRUCTURE analyses. PCA based on autosomal markers separated Greenland mallards into two clusters, one of which included a mallard from the British Isles (Fig. 2*A*). STRUCTURE further revealed that the Greenland mallards from the second cluster were admixed with North American and Eurasian mallards (Fig. 3*A*). Therefore, although there is no confirmed evidence from telemetry observations, banding records, or field sightings of the presence of *A. p. platyrhynchos* in Greenland or *A. p. conboschas* in Europe, the available data can be interpreted as indirect evidence of hybridization between the subspecies, probably resulting from overlapping migration routes and breeding grounds in the North Atlantic region. However, this question as well as the roles of genetic drift and selective pressure in Greenland's harsh environment in shaping the genetic profile of this taxon will undoubtedly be addressed in more detail through the larger-scale sampling of Greenland Mallard and by combining genetic information with a thorough comparative analysis of morphological traits.

Mallard and Eastern Spot-billed Duck

Despite the absence of divergence between North American and Eurasian Mallard populations based on Z-chromosome loci, there is some genetic differentiation between them at autosomal loci, which is characterized by subtle genome-wide shifts in allele frequencies rather than by strong divergence at specific loci. This cumulative and statistical signal emerges only when thousands of markers are analyzed together as demonstrated in the stable and distinct clusters recovered by STRUCTURE (Fig. 3*A*). Thus, North American and Eurasian wild mallards, while having different demographic histories, were shown to have no significant outlier loci in pairwise comparison (Lavretsky et al., 2023a: Fig. 4). This observed divergence may be interpreted in two ways. One possibility is that it represents an early stage of allopatric speciation with populations having become sufficiently distinct in their genetic backgrounds to be recognized as separate clusters. Alternatively, given the absence of outlier loci, this pattern may instead reflect a stochastic divergence accumulation via genetic drift and mutation. In this scenario, historical isolation has resulted in weakly differentiated yet statistically distinct demographic units that remain connected by sufficient gene flow to prevent the fixation of accumulating divergence.

Addition of the Eastern Spot-billed Duck to the analysis provides deeper insight into the Mallard lineage evolution in the Holarctic. Eastern Spot-billed Duck is a close relative of the Mallard and belongs to a group of 14 Mallard-like species with similar morphology, behavior, and genetics (Livezey, 1991; Johnson, Sorenson, 1999). Its breeding range spans East Asia from Siberia's Lake Baikal to southern Sakhalin, including Japan, Korea, and northeastern China. As a result of recent Eastern Spot-billed Duck range expansion, the two species' breeding areas now overlap across southeastern Siberia, the southern Russian Far East, northern Japan, and northeastern China, where they occasionally hybridize (Kulikova et al., 2004).

In the current study, after combining two ddRAD-seq datasets, we observe that the similarity of autosomal allele frequency spectra places mallards from Eurasia with the Eastern Spot-billed Duck in one cluster while grouping all North American mallards into the other as clearly visualized in the STRUCTURE barplot (Fig. 3*A*). Those subtle genome-wide shifts in allele frequencies differentiating Eurasian and North American mallards suggest that the Eastern Spot-billed Duck diverged from the Eurasian Mallard more recently than the initial split between the Palearctic and Nearctic Mallard populations. Demographic reconstructions based on whole genome sequencing support this. Specifically, a comparison of the PSMC trajectories from two recent studies – Lavretsky et al. (2023a: Fig. 4; North American Mallard, Eurasian Mallard, and Pekin duck) and Zhang et al. (2025: Fig. 3*B*; Eurasian Mallard, Eastern Spot-billed Duck, and Pekin duck) – shows that the North American Mallard lineage diverged earlier than the split between the Eastern Spot-billed Duck and the Eurasian Mallard. As for the somewhat lower differentiation between the Eastern Spot-billed Duck and the Mallard from Northeast Asia in contrast to pairwise comparisons involving other

Mallard populations, this is likely the result of historical and contemporary hybridization between these species in East Asia (Kulikova et al., 2004; Zhang et al., 2025). In support of this, approximately 20 phenotypic hybrids have been recorded in the Primorye region (southern Russian Far East) between 1964 and 2022 (Glushchenko et al., 2022; Kulikova et al., 2022, unpubl. data). Most of these hybrids exhibited intermediate plumage coloration, whereas two showed a phenotype closer to that of the Eastern Spot-billed Duck (Glushchenko et al., 2022; unpubl. data).

Nevertheless, while autosomal data clustered Eurasian mallards with Eastern spot-billed ducks, the Z-chromosome data clearly separated all spot-billed ducks into a distinct group in the population structure analyses (Figs 2*B* and 3*B*). Previously, using ddRAD-seq data we discovered 4.5 times higher genetic differentiation on the Z-chromosome than on autosomes and several fixed SNP differences on the Z-chromosome that can reliably discriminate between the two taxa (Kulikova et al., 2022). The whole genome sequencing revealed specific resistant to introgression genomic regions on Z-chromosome including a ~7.2 Mb area containing the sex-determination gene DMRT and a ~0.3 Mb region harboring the coloration gene SLC24A2, which displayed the highest differentiation (F_{ST}) between the two species (Zhang et al., 2025). However, no similar evidence has been found for autosomal regions. It can be hypothesized that these Z-chromosome islands of differentiation are precisely what maintain the species integrity of the Eastern Spot-billed Duck.

Within-population nucleotide diversity was depressed on the Z-chromosome across all Mallard and Eastern Spot-billed Duck populations, with the ratio of Z-chromosome to autosomal nucleotide diversity being much lower than expected from population size effects, ranging from 0.42–0.46 in Mallard populations to 0.33 in the Greenland Mallard and 0.23 in the Eastern Spot-billed Duck (Table S2). The previously estimated π_Z/π_A ratios, 0.40 for the Mallard and 0.32 for the Eastern Spot-billed Duck, were also considerably below the theoretical expectation of 0.75 and even below the theoretical minimum of 0.5625 expected under extreme variance in male reproductive success (Charlesworth, 2001). However, ducks are seasonally monogamous, and the frequency of extra-pair paternity (9.3 % in Mallards; Kreisinger et al., 2010) is not sufficient to explain such a pronounced reduction in Z-linked diversity. Thus, selection rather than demographic factors alone is most plausibly responsible for the observed contrast between Z-chromosome and autosomal nucleotide diversity.

A different picture of the relationships between the Mallard and the Eastern Spot-billed Duck emerges from earlier phylogeographic studies based on mitochondrial DNA (Kulikova et al., 2004, 2005). Two groups of mtDNA haplotypes (A and B) have been identified in the Mallard and other Mallard-group species across the Holarctic; they are estimated to have diverged around 400,000 years ago (Avise et al., 1990). Lavretsky et al. (2023b) clearly showed that haplogroup A is characteristic of the Eurasian Mallard while haplogroup B is native to North America. The high proportion of A haplotypes in modern North American mallards is not a natural phenomenon but a direct result of over a century of large-scale releases of game-farm mallards (which are of Eurasian domestic stock) leading to extensive introgression (Lavretsky et al., 2023b). Mallards across Eurasia predominantly possess A-group haplotypes, except for East Asia, where B-group haplotypes are found in both mallards and Eastern spot-billed ducks with A:B ratios of 19.5:1 and 1.6:1, respectively (Kulikova et al., 2004). These 'Asian' B haplotypes have been designated Spot-billed (SB) Ducks haplotypes and form a monophyletic subclade that diverges by 2–16 substitutions from the B haplotypes found in North American monomorphic Mallard-like species (Mottled, American Black, and Mexican Ducks), as well as in North American mallards. Phylogenetic reconstructions indicate that the SB and other B subclades have approximately similar ages, around 70,000 years, with a crown age of about 37,000 years (Fig. S3) suggesting a recent radiation. We assume that the Eastern Spot-billed Duck could have acquired the B-type mtDNA through hybridization with mallards from North America in the past while mallards from Northeast Asia could have acquired SB haplotypes through contemporary hybridization with Eastern spot-billed ducks. Ultimately, the mtDNA haplogroups under consideration trace the individual history of a single marker in several closely related and recently radiated Mallard-group species. Incomplete lineage sorting and hybridization limit the applicability of mtDNA in studies of phylogeny and speciation in the Mallard lineage. However, this marker could still be useful in tracing recent demographic processes and contemporary hybridization events.

Conclusion

Collectively, our findings illustrate a complex pattern of evolutionary outcomes in a recent avian radiation. They are ranging from a scenario where divergence remains incipient and primarily neutral to a case where reproductive isolation is due to sex-linked barriers or where divergence is driven by founder events. Specifically, we document (i) statistically distinct yet weakly differentiated units resulting from historical allopatric separation (Eurasian vs. North American Mallard populations), (ii) divergence-with-gene-flow involving sex-linked barriers (Mallard vs. Eastern Spot-billed Duck), and (iii) divergence driven by founder effects and adaptation to extreme environments, potentially leading to peripatric speciation (Greenland Mallard). However, we emphasize that these scenarios should be viewed as testable hypotheses rather than definitive conclusions. Formal demographic modeling and coalescent simulations will be required to rigorously discriminate among alternative evolutionary models.

This study clearly shows that unraveling such a complex evolutionary history requires combining autosomal, sex-linked, and mitochondrial markers across a broad geographic and taxonomic scale. We also believe that future studies of the Mallard species complex that comprises 13 extant species will definitely benefit from whole genome sequencing and resequencing data to more clearly determine the nature and evolutionary forces underlying diversification and stability of this syngameon, a "copulative community" of otherwise distinct species that are connected by frequent or occasional hybridization (Buck, Flores-Rentería, 2022). The significant genetic distinctiveness of the Greenland Mallard remains a no-

table question. We suggest that its investigation should involve morphological analysis to complement genetic data, satellite tracking to investigate its migratory patterns and connectivity, and whole genome resequencing to identify genomic regions driving local adaptation.

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