

Genetic Insights into the Biogeography and Evolutionary History of the Killer Whale

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Abstract

This paper reviews genetic studies undertaken to generate an accurate phylogeny of killer whale (*Orcinus orca*) lineages. Three phylogenies, distinct in branching order and timing of divergences, are discussed. Of the three phylogenies, two are based on mitochondrial DNA and show more ancient divergence times for killer whale lineages. The third phylogeny, based on nuclear DNA, indicates that killer whales diversified more recently. The differing phylogenies have differing implications for biogeographic inference and for species assessments. The nuclear based phylogeny suggests that killer whale lineages diverged in sympatry while the mitochondrial based phylogenies as well as an empirical study indicate that the divergences more likely occurred in allopatry. The mitochondrial based phylogenies also support revision of killer whale taxonomy as there is indication that killer whales constitute multiple species and subspecies instead of a single species. The types of research that would be helpful in confirming hypotheses based on the phylogenies are discussed.

Introduction

The existence of pronounced morphological and behavioral variation among modern killer whales (*Orcinus orca*) has been well documented for several decades and has led researchers to delineate different “types” or “ecotypes” of killer whales (1). Further study of these ecotypes has prompted two pressing questions to arise in the field. The first asks whether the divergences of these unique ecotypes occurred in sympatry and thus represent an exceedingly rare evolutionary phenomenon. The unrelated second question asks whether the differences present between ecotypes are sufficient to warrant elevation of several ecotypes to full species status. Distinguishing two species from each other is important as it could facilitate proper conservation effort (2). Recently, genetic studies have attempted to reveal the evolutionary and biogeographic history of the ecotypes in order to answer these questions. Different sources of DNA samples (i.e. mitochondrial versus nuclear DNA) as well as sample size and length of DNA sequences used has led to the creation of disparate phylogenies that differ in their branching orders and times of divergence. This review article will evaluate the varying success that studies have had in creating an acceptable phylogeny for killer whales as well as discuss how the different phylogenies affect answers to the two questions presented above.

Modern Killer Whale Types and Geographic Ranges

There are three locations where killer whales and their ecotypes are most heavily studied: the North Pacific, the North Atlantic, and the Southern Ocean (1). In the North Pacific, killer whales are categorized into three ecotypes: residents, transients, and offshores. In the North Atlantic, two ecotypes are present: Type 1 and Type 2. The killer whales of the Southern Ocean constitute five ecotypes: Type A, Type B (large), Type B (small), Type C, and Type D. Each ecotype, in addition to having a unique social structure, is morphologically unique (1). Furthermore, each ecotype has a distinct prey specialization which causes some ecotypes to be more directly impacted by certain human actions than others (2). The tables below summarize the traits characteristic to each ecotype.

Table 1. A summary of the ecotypes of the Northern Hemisphere.

Ecotype	Proposed common name	Morphology	Diet	Distribution
Resident (ENP)	Resident killer whale	Tall dorsal fin, may be forward slanted, often has very open saddle, dorsal fin rounded on top	Fish and squid	ENP - spanning much of the central west coast of North America
Transient (ENP)	Bigg's killer whale	Typically pointed dorsal fin, eyepatch slants slightly downward towards the rear, closed saddle, often extends past midline of dorsal fin	Cetaceans and pinnipeds	
Offshore (ENP)	Offshore killer whales	Faint saddle patch, often rounded dorsal fin at tip, often with nicks in dorsal fin	Bony and cartilaginous fishes	
Type 1 Eastern North Atlantic		Conspicuous saddle patch, relatively large eyepatch	Mainly fish but some pinnipeds	North Atlantic (bordered in the south by Norway and Iceland)
Type 2 Eastern North Atlantic		Faint saddle, patch often slants towards rear	Mainly cetaceans	

Table 2. A summary of the ecotypes of the Southern Hemisphere.

Ecotype	Proposed common name	Morphology	Diet	Distribution
Type A	Antarctic killer whale	All black, large killer whale, medium-sized eye-patch parallel to body, females occasionally have slightly open saddle, size range 7–9 m	Cetaceans and seals	Open water right around Antarctica in summer; winter distribution unknown
Type B (big)	Pack ice killer whale	Two-tone grey, large grey dorsal cape, eyepatch variable but larger than other whales, often yellow with diatoms	Pinnipeds	Pack ice in summer; unknown in winter
Type B (small)	Gerlache killer whale	As above but half the size	Penguins	Pack ice in summer, largely Antarctic Peninsula; winter tropical
Type C	Ross Sea killer whale	Two-tone grey and white, smallest reaching only 6 m in length, eyepatch angled at 45° to body	Fish	Antarctic pack ice; winter unknown, possibly remain all year round
Type D	Sub-Antarctic killer whale	Extremely small eyepatch and bulbous head	Unknown	Sub-Antarctic and temperate waters

Generating an Accurate Phylogeny: Mitochondrial versus Nuclear DNA

The analysis of mitochondrial, over nuclear, DNA is most commonly employed in studies aiming to infer an accurate phylogeny (3) as it is generally seen as providing more clarity to evolutionary relationships due to the fact that mitochondrial DNA is passed down through maternal lineages (2). The killer whale, however, exhibits, like most cetacean species, very low genetic diversity in the mitogenome. This makes it difficult to use the analysis of mitochondrial DNA in the generation of reliable phylogenetic trees (2). Despite the many measures taken by researchers to create accurate phylogenies, there are numerous discrepancies between the phylogenies published by different studies.

Introduction to Recently Hypothesized Phylogenies

Of recently hypothesized phylogenies, the phylogeny showing the deepest divergences is based on data from entire mitogenomes of 139 killer whales (2). The phylogeny estimates that the most recent common ancestor (MRCA) of all modern whales lived about 700,000 years. This suggests that the first bifurcation in the killer whale lineage occurred at this point in time when the transient lineage diverged. The MRCA of all other killer whales excluding the transients is estimated to have lived 300,000 years ago, at which point the Antarctic killer whale lineage diverged. Within this Antarctic lineage, diversification into the ecotypes of Antarctic today occurred between 151,000 and 59,000 years ago. The remaining lineage would eventually diversify into the current killer whale types of the Northern Hemisphere excluding the transients but including Type 1, Type 2, the residents, and the offshores. These divergence events are estimated to have occurred between 188,000 to 80,000 years ago (2). This phylogeny structure, characterized by its ancient divergence times, was replicated to a certain extent in another study that utilized mitogenomes (5).

However, shallower trees with more recent divergence times and different branching orders have also been constructed. The shallowest hypothesized phylogeny is based on data obtained from nuclear DNA (4). This tree shows that the MRCA of all killer whales was very recent and likely lived only 189,000 years ago (4). Besides its strikingly recent divergence times, this phylogeny is also characterized by its unique branching order. At the phylogeny's most basal node is not the transient lineage but the Antarctic lineage. The transient lineage divergence came afterwards, followed by the divergence of the North Atlantic lineages and finally the bifurcation of the resident and offshore lineages. Compared to mitochondrial DNA phylogenies, this nuclear DNA phylogeny is notable in the fact that the offshore and North Atlantic lineages are monophyletic as well as the fact that the residents and the offshores are more clearly divided into two separate lineages (4).

A tree with an estimate of the time to the MRCA falling in between the more ancient and recent estimates has also been published (6). This phylogeny suggests the time to the MRCA of all killer whales is 360,000 years. In terms of branching order, it is identical to those mitochondrial phylogenies described above that suggest a much more ancient time to the MRCA. However, the tempo of the diversification hypothesized by this phylogeny is unique in that it is very rapid. Shortly after the MRCA 360,000 years ago, all killer whale lineages rapidly diverged at approximately the same time into the lineages that would lead into the ecotypes of today (6). There is reason to believe this hypothesized phylogeny is superior in its accuracy. With DNA sequences from an impressive 452 killer whale individuals (6), this phylogeny is based off the largest sample size of all phylogenetic studies on killer whales to date. Additionally, the DNA samples analyzed were obtained from killer whale individuals in low population density areas (most sample collections have historically taken place in high population density areas) and at sites more representative of populations' full ranges (6). The type of DNA utilized to generate the phylogeny was primarily mitochondrial (full mitogenomes were used) but regions of nuclear DNA were used to supplement the mitochondrial DNA (6). Despite the robust methods taken in

its generation, further study is still necessary to confirm the accuracy of this phylogeny and all other published phylogenies.

Biogeographic Analyses and Mode of Divergence

How speciation occurs in ocean waters has been somewhat of a paradox to researchers. Unlike terrestrial environments, oceanic environments, for the most part, lack physical barriers that induce isolation and reduce gene flow (7). The paradox is compounded when considering highly mobile marine species like killer whales (6). Thus, it is of great interest to researchers to determine how killer whale ecotypes that currently have sympatric, overlapping ranges diverged. Due to the contradictory results that are produced when biogeographic models are applied to the differing phylogenies described above, two conflicting theories specifically related to the divergence North Pacific ecotypes have developed in the literature.

A divergence in sympatry, a rare mode of evolution, for the North Pacific ecotypes is most supported by the nuclear DNA phylogeny described above. When the software RASP (Reconstruct Ancestral State in Phylogenies) was applied to the nuclear DNA based phylogenetic tree, the inferred historical biogeography of killer whale populations indicated that some divergences did occur in the same ocean basins (4). Specifically, the most supported biogeographic scenario suggests that killer whales dispersed from an ancestral population in the Southern Ocean to the Pacific Ocean. Within the Pacific Ocean, the transient lineage diverged from other Pacific Ocean populations. After this divergence event, there was a dispersal of some individuals to the North Atlantic via the Northwest Passage; these individuals would found all future North Atlantic populations. The remaining North Pacific populations (excluding the already diverged transient populations) then diverged into the offshore and resident lineages (4). Of course, there is no way to verify that divergences actually occurred in sympatry even if populations inhabited the same ocean basin. The ranges of the three North Pacific ecotypes overlap today, yet the extent of historical overlap of the ranges in the North Pacific cannot be conclusively determined. Still, the ranges of these highly mobile marine predators would have likely overlapped if populations occupied the same ocean basin (4).

Others researchers contend that the historical biogeography of the killer whale actually contained periods of allopatry during which some populations diverged when were isolated from each other. When an Isolation with Migration Analysis (IMa) was applied to the mitochondrial DNA phylogeny with the deepest divergence times described above, the best supported biogeographic history indicated that there were many migrations to and from different ocean basins (5). There was bi-directional movement between the ocean basins, meaning that killer whale lineages traveled from the Pacific to the Atlantic as well as from the Atlantic to the Pacific. It is argued that some of these migrations resulted in reproductive isolation of certain killer whale populations which eventually led to divergence (5). This exact pattern of migration and dispersal was not shown in a more recent study that applied the RASP software to the mitochondrial DNA tree with an MRCA living 360,000 years ago (6). However, this newer study did show several cases of interoceanic and interhemispheric movements (some of which were quite recent) that also support that killer whale divergences occurred in allopatry. The current

condition of sympatry of killer whale populations observed in the North Pacific, North Atlantic, and Southern Ocean is merely secondary contact, achieved through recent migrations, of previously diverged lineages (6).

As of yet, there has been no reconciliation between these two contradictory theories. Proponents of the theory that divergences occurred in sympatry (a theory based on the use of nuclear DNA phylogeny) argue that using a mitochondrial DNA phylogeny to infer the movements of populations could be misleading as mitochondrial DNA tracks only maternal lineages (8). Additionally, the patterns observed in the mitogenome of killer whales could be obscured by a widespread historical bottleneck event (8) suggested to have occurred by some (9,10) but not all (6) studies. On the other hand, proponents of the theory that divergences occurred in allopatry (a theory based on the use of a mitochondrial DNA phylogeny) assert that nuclear DNA is inadequate when used to make biogeographic inference because secondary contact of previously allopatric ecotypes has allowed for inter-ecotypes matings to occur (11). While the extent of inter-ecotype matings is not known for all the killer whale ecotypes, a study did show that there are low-levels of continual gene flow between the three North Pacific ecotypes, particularly the transients and offshores (12). These inter-ecotype matings during which a male temporarily disperses from his pod to mate with a female of another ecotype (12) could distort the patterns observed in the nuclear DNA and could lead to inaccurate biogeographic inference (11). Further studies that investigate the current levels of gene flow between currently sympatric populations would be very valuable in order to evaluate the power that mitochondrial versus nuclear DNA based phylogenies have to make accurate biogeographic inferences. Another valuable type of study to conduct would be one that empirically tests the feasibility of sympatric divergence in killer whales. To date, one study has attempted to undertake this task by comparing lineage sorting with the extent of niche specialization (assessed through dietary habits inferred from stable carbon and nitrogen isotope composition analysis) in North Atlantic killer whale subfossils as well as living North Atlantic killer whale individuals (13). The results indicate that despite strong historic and current evidence for niche specialization, divergence of lineages with different niches did not occur to a significant degree nor is it appearing to occur today because of ongoing gene flow among killer whales with different niches (13). Thus, this empirical study of North Atlantic killer whales calls the practicality of sympatric divergence of killer whale ecotypes into question.

Has Speciation Occurred?

Regardless of whether the divergences between certain killer whale types occurred in sympatry or allopatry, the unrelated question of whether ecotypes are divergent enough to be considered multiple species of killer whales still remains. The most significant benefit to differentiating killer whale ecotypes into full species or subspecies (if there is adequate supporting evidence) is that it may support appropriate conservation efforts (2). Different ecotypes are exposed to differing levels of human-caused prey depletion as well as differing

levels of human-caused bioaccumulation of toxic compounds (2) and thus require unique conservation approaches.

Noting the genetic differences between some lineages, in addition to the sheer length of time that lineages have been divergent for (as shown on the mitochondrial DNA based tree that posits that the MRCA of all killer whales lived 700,000 years ago), researchers have recommended that several amendments be made to killer whale taxonomy (2). There is indication that the transient ecotype is so distinct that the transients should be elevated to full species status. Also substantially divergent from all other killer whale types are the Antarctic types. Of the Antarctic types, Type B, Type C (2), and Type D¹ (14) are divergent enough to be elevated to three unique species. There is not strong evidence to justify elevation of the remaining ecotypes (offshores, residents, Type 1, Type 2, and Type A) to full species status but an argument can be made that they should be considered subspecies of the same killer whale species (2).

In recent years however, the strength of these arguments for the amendments presented above has waned as phylogenies with different branching orders and dates of divergence have been generated. Nuclear phylogenies have not confirmed (4) the lineage sorting patterns that appear in the mitochondrial based phylogenies that researchers used to make the species status assessments described above. Furthermore, a 2011 study recorded inter-ecotype breeding between the three ecotypes of the North Pacific (12) indicating that the North Pacific ecotypes would not qualify as separate species under the biological species concept (BSC). Regrettably, no further articles have been published to prove or disprove the idea that killer whales represent multiple species as attention within the field seems to have shifted to the testing the occurrence of sympatric divergence between killer whale types. Regardless of whether the different whale ecotypes qualify as separate species or subspecies, conservation efforts must be tailored to the different ecotypes. While a proper species classification would certainly aid conservation efforts, making informed decisions on the conservation of killer whales ultimately will not come from consulting arbitrary species distinctions but from tapping into the knowledge gained from ecological studies.

Conclusions

Molecular genetic studies have confirmed that modern killer whale ecotypes are not only morphologically and behaviorally distinct but also genetically distinct. However, estimates for when the ecotype lineages diverged from a common ancestor is still a matter of contention. The mitogenomes of killer whales worldwide have low genetic diversity (2), making it difficult to infer evolutionary relationships. The killer whale phylogenies published vary in their time to a MRCA, branching order, and tempo of divergences. Typically, mitochondrial DNA based phylogenies suggest deep divergence dates for killer whale ecotypes while nuclear DNA based

¹ Note that Type D (sometimes considered a sub-Antarctic killer whale type) diverged not from the lineage that eventually diversified into all other Antarctic ecotypes but from the transient lineage an estimated 360,000 years ago(14).

phylogenies indicate the opposite. The differing phylogenies have created several scenarios of historical biogeography with different implications for mode of divergence (sympatry versus allopatry) for the ecotypes. Additionally, some phylogenies have supported the elevation of four ecotypes (transients, Type B, Type C, and Type D) to full species status. Further studies in this field will be vital to resolve the many uncertainties that remain. Specifically, additional genetic studies are required to reconcile the differences in mitochondrial and nuclear based phylogenies. Empirical studies are also needed to further confirm the unlikelihood of sympatric divergences. Lastly, genetic studies specifically focusing on evidence for or against speciation would be beneficial, as would large-scale ecological studies on all killer whale types to confirm speciation assertions made based on genetic data.

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