Evolutionary Ecology Research, 2012, **14**: 447–465

Investigating ecological speciation in non-model organisms: a case study of killer whale ecotypes

Andrew D. Foote

Centre for GeoGenetics, Natural History Museum of Denmark, Copenhagen, Denmark

ABSTRACT

Background: Studies of ecological speciation tend to focus on a few model biological systems. In contrast, few studies on non-model organisms have been able to infer ecological speciation as the underlying mechanism of evolutionary divergence.

Questions: What are the pitfalls in studying ecological speciation in non-model organisms that lead to this bias? What alternative approaches might redress the balance?

Organism: Genetically differentiated types of the killer whale (*Orcinus orca*) exhibiting differences in prey preference, habitat use, morphology, and behaviour.

Methods: Review of the literature on killer whale evolutionary ecology in search of any difficulty in demonstrating causal links between variation in phenotype, ecology, and reproductive isolation in this non-model organism.

Results: At present, we do not have enough evidence to conclude that adaptive phenotype traits linked to ecological variation underlie reproductive isolation between sympatric killer whale types. Perhaps ecological speciation has occurred, but it is hard to prove. We will probably face this outcome whenever we wish to address non-model organisms – species in which it is not easy to apply experimental approaches and comparative studies among multiple taxon pairs. We need new genomic approaches that identify genes under selection and then link alleles to phenotypic differences and reproductive isolation.

Keywords: ecological speciation, genome-wide scans, killer whale, niche variation, non-model organism, phenotypic variation, reproductive isolation.

INTRODUCTION

Ecological differences have long been recognized as having a key role in promoting speciation through natural selection (Darwin, 1859). Early studies on speciation were primarily descriptive and species were typically categorized based on phenotypic traits. Subsequently, reproductive isolation rather than simply phenotypic divergence came to be recognized as the critical indicator of speciation (Dobzhansky, 1937; Mayr, 1942). Modern studies use the term 'ecological speciation' to refer to a three-stage process that encompasses and links

---

Correspondence: A.D. Foote, Centre for GeoGenetics, Natural History Museum of Denmark, Øster Volgade 5–7, Copenhagen K, DK-1350, Denmark. e-mail: footead@gmail.com

ecological divergence, phenotypic divergence, and reproductive isolation: ecological contrast or an ecological gradient promotes divergent natural selection; this causes adaptive divergence of phenotypic traits between individuals in the different ecological contexts; when one or several of these adaptations are also associated with reproductive compatibility, divergence in these traits results in reproductive isolation either through assortative mating or low hybrid fitness (Schluter, 2001, 2009; Rundle and Nosil, 2005; Funk *et al.*, 2006; Hendry, 2009a, 2009b; Nosil *et al.*, 2009). Therefore, robustly determining that ecological speciation is the underlying mechanism of any evolutionary divergence requires each of the following steps to be achieved:

1. The identification of ecological differences between speciating taxa.
2. The identification of adaptive divergence due to natural selection of phenotypic traits associated with these ecological differences.
3. Determining that these diverging taxa are reproductively isolated.
4. The identification of a causal link between the adaptive traits under natural selection and reproductive isolation.

A series of studies on model systems has led to the development of a framework of investigations, including investigating parallel divergences, translocation experiments, and mate-choice experiments, with which to robustly test if observations of ecological, phenotype, and genetic variation are consistent with ecological speciation (e.g. Schluter and Nagel, 1995; Orr and Smith, 1998; Schluter, 2001; McKinnon and Rundle, 2002; Hendry, 2009a; Nosil *et al.*, 2009). These investigations into ecological speciation have led to the identification of the underlying mechanisms linking natural selection and reproductive isolation in some well-studied natural model systems, e.g. Darwin's finches *Geospiza* spp., threespine sticklebacks *Gasterosteus aculeatus*, and Anolis lizards *Anolis sagrei*, to name a few (McKinnon and Rundle, 2002; Grant and Grant, 2008; Losos, 2009).

Ecological speciation in non-model organisms

The recent high level of interest in ecological speciation has led to investigations exploring its prevalence in a broader range of taxa (e.g. Funk *et al.*, 2006). However, studies robustly identifying ecological speciation in non-model systems and determining the underlying mechanisms are still relatively few and some studies have over-interpreted ecological, phenotypic, and neutral genetic differences between populations as conclusive support for ecological speciation (see Hendry, 2009a). There are arguably understandable reasons for why investigations on non-model systems might have difficulty establishing the causal links between ecological differences, selection on adaptive traits, and reproductive isolation, needed to robustly infer ecological speciation. For example, many non-model organisms are long-lived, making it unviable to track evolution across multiple generations. There can be ethical and logistical constraints on experimental work such as translocations and mate-choice experiments. There can also be difficulty in finding multiple taxon pairs to allow comparisons of parallel evolution. However, studying ecological speciation and understanding the processes underlying reproductive isolation between putative species or 'evolutionarily significant units' is now an important and widely accepted aspect of conservation genetics (see Crandall *et al.*, 2000). Ecological speciation is, after all, a key component of the generation of biodiversity. Therefore, we should not give up on our efforts to broaden investigations of ecological speciation to a wider range of taxa.

Top-down and bottom-up approaches

Most studies of ecological speciation have adopted what Schluter (2009) referred to as a top-down approach – that is, first identifying phenotypic traits under divergent selection due to ecological differences (e.g. Nagel and Schluter, 1998); second, identifying which of these traits are associated with reproductive isolation (e.g. McKinnon *et al.*, 2004); and lastly, understanding the underlying genes associated with those traits and reproductive isolation (e.g. Peichel *et al.*, 2001). However, the advent of high-throughput sequencing methods and growing ease and accessibility of genomic approaches such as genome-wide scans through RAD-sequencing (Baird *et al.*, 2008; Elshire *et al.*, 2011) allow for more widespread application of a bottom-up approach (*sensu* Schluter, 2009), i.e. identifying outlier loci for which allele frequencies differ between ecotypes more than expected under a neutral model, suggesting evolution under positive selection due to being associated with adaptive divergence. This can then be followed by mapping these genes under selection to phenotypic traits, and an investigation of how alternative alleles in those genes are expressed as phenotypic differences, and lastly how they are linked to reproductive isolation. For example, recent studies have found parallel divergences in loci linked to armour plate phenotype, salinity tolerance, and immune function in different populations of marine–freshwater species pairs, and in loci linked to pigmentation and immune function in multiple benthic–limnetic lake pairs of threespine stickback (Hohenlohe *et al.*, 2010; Jones *et al.*, 2012). Using this approach, studies on model and non-model organisms should be on more of an equal footing, as experimental work, multi-generational studies or comparisons of multiple taxon pairs are not required to initially identify adaptive differences.

KILLER WHALES AS A NON-MODEL ORGANISM FOR STUDYING ECOLOGICAL SPECIATION

Here, I first adopt a top-down approach to critically review the evidence that distinct ecotypes of a non-model organism, the killer whale *Orcinus orca*, are a product of ecological speciation, as has recently been suggested by Riesch *et al.* (2012). I then outline how a bottom-up approach might help resolve some of the uncertainties and result in a more conclusive assessment.

Is there evidence for ecological divergence?

The best-studied populations of the Northeast Pacific exhibit strong ecological contrast: populations of the 'transient' ecotype specialize in hunting mammals and occur in partial sympatry with populations of the 'resident' ecotype, which specialize in hunting fish, in particular salmonids (Ford *et al.*, 1998; Saulitis *et al.*, 2000; Burdin *et al.*, 2005; Herman *et al.*, 2005) (Fig. 1). A third Pacific 'offshore' ecotype is thought to be pisciverous, with a diet that includes shark species (Dahlheim *et al.*, 2008; J.K.B. Ford *et al.*, 2011).

In the waters around Antarctica, several discrete morphotypes (types A, B, C, and D) have recently been described (Pitman and Ensor, 2003; Pitman *et al.*, 2007, 2011) (Fig. 1). Studies on the ecological differences between Antarctic types are at an earlier stage than studies in the Northeast Pacific, but suggest types A and D mainly inhabit open waters, while types B and C are more often found in the pack ice (Pitman and Ensor, 2003; Andrews *et al.*, 2008; Pitman and Durban, 2010, 2012; Pitman *et al.*, 2011; Durban and Pitman, 2012). Furthermore, while diets may not be exclusive

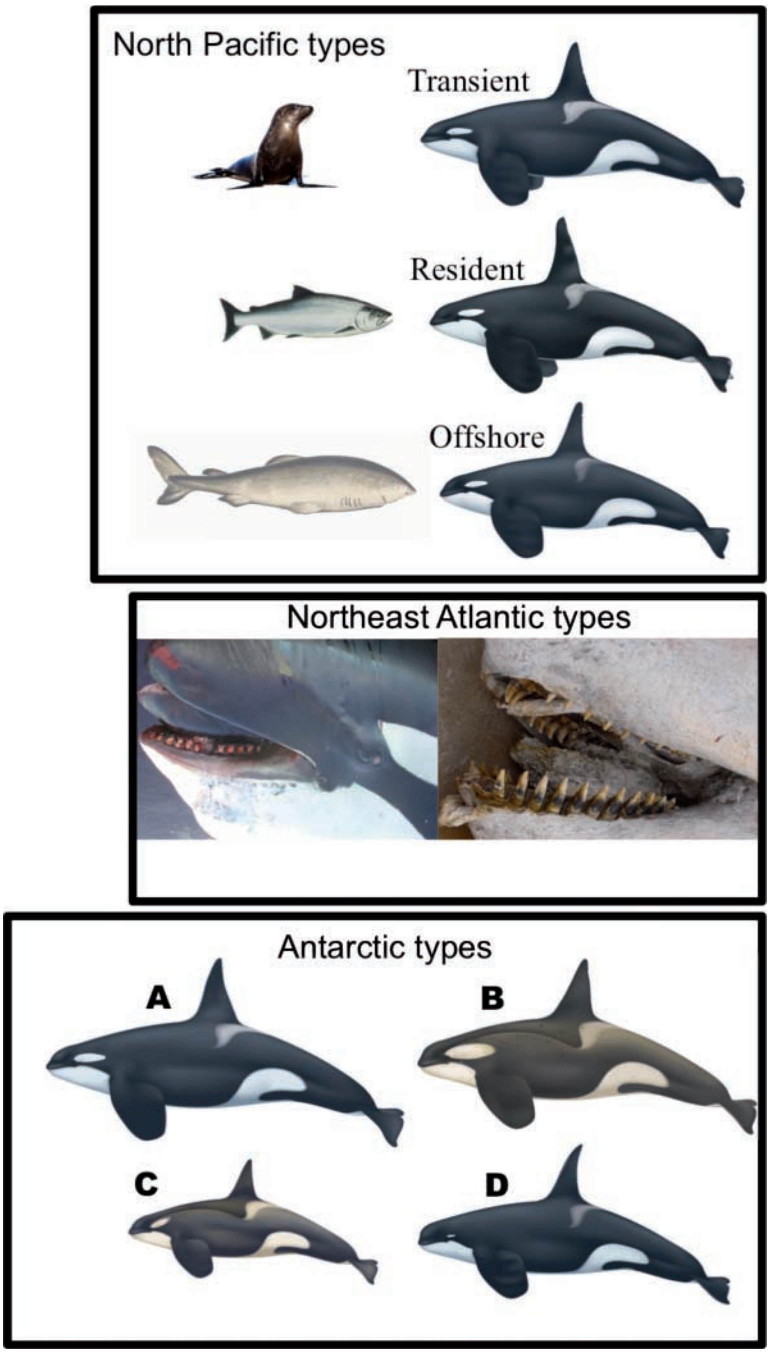

Fig. 1. Phenotype variation among killer whale types (for details, see main text). Illustrations of adult male killer whales drawn to scale by Uko Gorter based on published (Pitman *et al.*, 2007; Fearnbach *et al.*, 2011) and unpublished data collected by John W. Durban and Robert L. Pitman.

to each type, there does appear to be differences in the dominant prey that each type has been observed hunting: type A have mainly been observed hunting minke whales, type B seals and penguins, and type C Antarctic tooth fish (Pitman and Ensor, 2003; Pitman and Durban, 2010, 2012). Recent studies suggest there is potentially more variation in both morphology and ecology within type B than previously recognized, and there may be a smaller penguin-eating form and a larger seal-eating form (Pitman and Durban, 2010, 2012).

In the Northeast Atlantic, two types were recently described based on tooth wear patterns in museum samples (Foote *et al.*, 2009). Adult specimens of one type had severely worn teeth, while adult specimens of the other had little or no apical tooth wear (Fig. 1). The specimens with worn teeth shared mitochondrial DNA haplotypes with local populations known to feed on herring and mackerel, and tooth wear was seen in the wild in killer whales in these populations. Comparatively little is known about the ecology of the type with unworn teeth, but invariant stable isotope values suggest a specialized diet (Foote *et al.*, 2009). Interestingly, in the type with worn teeth, a large variance in stable isotope values indicates a broad niche width within each lineage (individuals sharing mtDNA haplotype). Figure 2 compares the standard deviation in isotopic values for one of these lineages with isotope values from both the mammal-eating and fish-eating types of the Pacific. The variance appears to indicate trophic level differences within a lineage. However, the presence of tooth wear in individuals at both ends of the scale suggests some overlap in diet or foraging strategy (Foote *et al.*, 2009) (Fig. 2). There have been a few observations of photo-identified groups from herring-eating populations hunting seals off the coast of Norway (Stenersen and Similä, 2004) and stomach contents of museum specimens also contained mammals (Foote *et al.*,

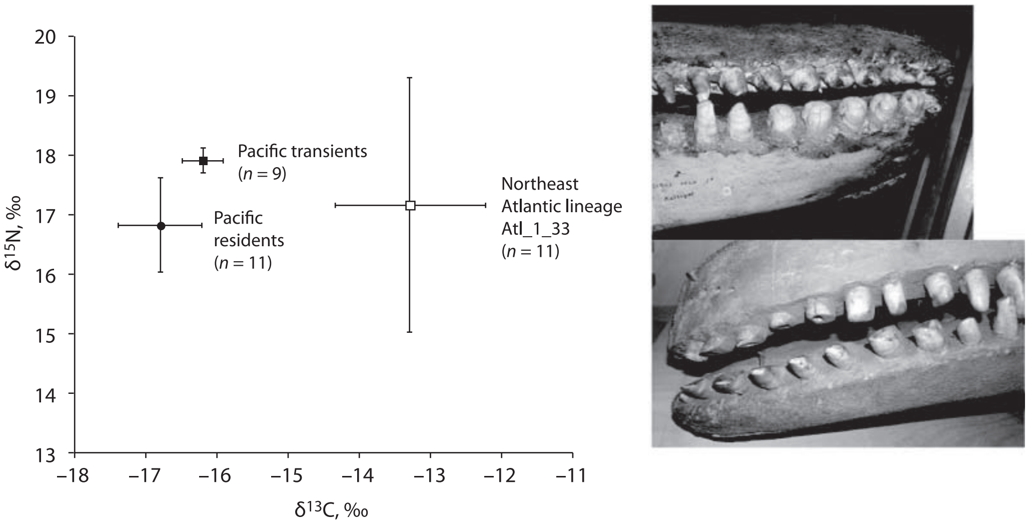

Fig. 2. Nitrogen and carbon stable isotope ratios of epidermal collagen samples for Pacific transient and resident killer whales from the Eastern Aleutian Islands in the North Pacific (Herman *et al.*, 2005), and bone or tooth collagen samples for a northeastern Atlantic killer mtDNA lineage (Atl_1_33) from the North Sea (Foote *et al.*, 2009). The worn teeth of northeastern Atlantic killer whales with low $\delta^{15}N$ values (e.g. bottom left whale has $\delta^{15}N = 12.8$) and high $\delta^{15}N$ values (e.g. top left whale has $\delta^{15}N = 16.1$ and had marine mammal remains in its stomach) suggest some overlap in the diet (Foote *et al.*, 2009).

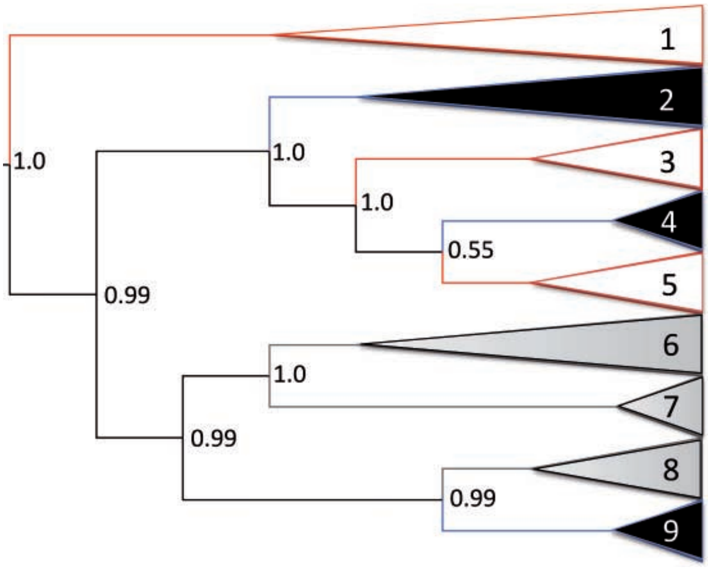

Fig. 3. Phylogenetic reconstruction based on mitochondrial genome sequences, adapted from Foote *et al.* (2011a). Clades are collapsed and colours indicate geographic origin of samples as follows: Antarctic (grey), Atlantic (black), and Pacific (white). The predominant ecotype or type in each clade is as follows: clade 1, Pacific transient; clade 2, Atlantic haplotypes from the Icelandic and Norwegian herring-eating populations and Gibraltar tuna-eating population; clade 3, Pacific resident; clade 4, Atlantic haplotypes from UK, Canary Islands, and Iceland; clade 5, Pacific offshore; clade 6, Antarctic type C; clade 7, Antarctic type B; clade 8, Antarctic type A; clade 9, Atlantic haplotypes from Scotland, Canary Islands, and Gulf of Mexico.

2009). Therefore, there is ecological contrast both between and within some lineages in the Northeast Atlantic.

Based on phylogenetic analyses, the ecological divergences listed above in the North Pacific, Northeast Atlantic, and Antarctic appear to have arisen to some extent independently in each ocean basin (Morin *et al.*, 2010; Foote *et al.*, 2011a) (Fig. 3). Divergent types in the North Pacific and Northeast Atlantic appear to have existed in allopatry prior to their current sympatric existence following secondary contact (Foote *et al.*, 2011a). The Antarctic types sequenced to date are sister taxa and so may have diverged within this region (Morin *et al.*, 2010; Foote *et al.*, 2011a). However, the extent of the Southern Ocean prohibits inferring sympatric divergence from biogeography alone (Foote *et al.*, 2011a). The divergence in stable isotopes of individuals from a localized geographic area within the Northeast Atlantic sharing the same mitochondrial DNA haplotype could suggest a partially sympatric diversification (Foote *et al.*, 2009).

In summary, there is evidence for ecological differences between killer whale types in the North Pacific, Antarctic, and Northeast Atlantic. However, the exact nature of ecological diversification and the extent of ecological discreteness are different in each ocean basin.

Is there evidence for adaptive divergence due to natural selection on phenotypic traits associated with ecology?

Natural selection can act on, and lead to divergence of, both genetically heritable traits and plastic traits (Crispo, 2008). The relationship between selection, adaptation (genetic and plastic), and reproductive isolation can be complex and adaptation of plastic traits can both constrain and/or promote progress towards ecological speciation. The key difference between these two forms of adaptive divergence is that while the divergence of heritable traits will be constrained by gene flow, plastic traits are directly influenced by ecological factors and unaffected by the levels of gene flow (Crispo, 2008; Thibert-Plante and Hendry, 2011). Plasticity can therefore lead to rapid adaptation to novel ecological niches, and this dispersal potential can reduce genetic differentiation across ecological gradients (Crispo, 2008; Thibert-Plante and Hendry, 2011). However, the ability to rapidly adapt to novel ecological niches through plasticity can then promote adaptive divergence of the genotype due to natural selection and therefore promote ecological speciation (Crispo, 2008; Thibert-Plante and Hendry, 2011). It is therefore important to distinguish between plastic and heritable traits when identifying adaptive divergence.

A key predictor for testing if the divergence of phenotypic traits is adaptive due to natural selection is if parallel evolution of phenotype divergence occurs across the same ecological gradient independently in multiple taxon pairs (Schluter, 2001). Due to the differences in ecological niche partitioning and phenotypic divergence, these three sets of ecotypes in the North Pacific, Northeast Atlantic, and Antarctic do not appear to be completely homologous in the same way as the parallel divergences in many model organisms are, such as the multiple benthic–limnetic lake threespine stickleback species pairs (McKinnon and Rundle, 2002). Instead, they appear more akin to the different systems within the threespine stickleback species complex (see McKinnon and Rundle, 2002). This confounds comparative investigations of parallel evolution. The different populations of the resident and transient ecotype found around the North Pacific Rim appear to have arisen from a single divergence rather than multiple parallel divergences, further reducing the opportunity for comparative studies. This is also probably the case in the other ocean basins, based on the almost complete monophyletic lineage sorting of each type (Morin *et al.*, 2010). However, recent comparative studies in the Northeast Atlantic have been able to draw useful parallels with results from the North Pacific (Deecke *et al.*, 2011; Beck *et al.*, 2012).

One of the strongest examples of an adaptive phenotypic trait linked to ecology in killer whales is vocal behaviour, which contrasts between groups hunting mammals and those hunting fish (Morton, 1990; Barrett-Lennard *et al.*, 1996; Deecke *et al.*, 2005, 2011). Marine mammals such as porpoise and seals have hearing ranges that overlap with the frequency range of vocalizations produced by killer whales, whereas most fish species have relatively poor hearing and over a narrow bandwidth (Foote and Nystuen, 2008). The cost of vocalizing by killer whales hunting mammals has been experimentally demonstrated by playing back killer whale calls to seals, which respond in a way that could reduce the risk of capture (Deecke *et al.*, 2002). Consequently, in the Northeast Pacific, the mammal-eating transients rarely vocalize when they are hunting, in contrast to the fish-eating resident type, which frequently vocalizes when foraging (Morton, 1990; Barrett-Lennard *et al.*, 1996; Deecke *et al.*, 2005). The risk of being detected when hunting mammals has also led to the evolution of consistently small group sizes in mammal-eating killer whales (Morton, 1990; Baird and Dill, 1996). In contrast, in fish-eating killer whales, group size varies markedly (Morton, 1990). Both these traits, vocal rates and group

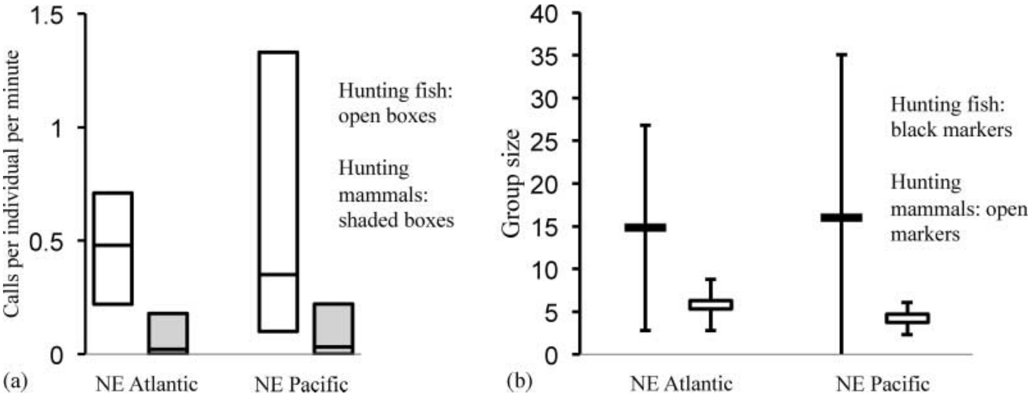

Fig. 4. Parallel divergence in phenotype traits associated with ecology. (a) Differences in the rate of vocalization by killer whales observed hunting either mammalian or fish prey types in the Northeast Pacific and Northeast Atlantic. Horizontal bars give median call rate, boxes show the interquartile range. Adapted from Deecke *et al.* (2005, 2011). (b) Differences in group size (mean ± 1 s.d.) between killer whales observed hunting either mammalian or fish prey types in the Northeast Pacific and Northeast Atlantic. Adapted from Beck *et al.* (2012) and Zerbini *et al.* (2007).

size, show a strong parallel contrast between killer whale groups hunting mammals and killer whale groups hunting fish in the Pacific and Atlantic, suggesting parallel evolution of adaptive traits due to natural selection (Deecke *et al.*, 2011; Beck *et al.*, 2012) (Fig. 4).

Although there is variation in body size between the Pacific types, to date no variation in genetically heritable traits have been shown to be due to ecological adaptation. Differences in morphological traits such as pigmentation patterns and body size are more pronounced among Antarctic types than Pacific types (Pitman and Ensor, 2003; Pitman *et al.*, 2011) (see Fig. 1). The degree of divergence in a phenotypic trait when compared with phylogenetic divergence can indicate the rate of divergence in these traits and therefore act as a proxy of the strength of natural selection (Barraclough and Nee, 2001; Schluter, 2001; Nosil *et al.*, 2009). Given the more recent phylogenetic divergence of the Antarctic types, it is hard to imagine that the morphological differences between them arose solely from drift. However, these variable traits have yet to be shown conclusively to be ecologically adaptive.

Natural selection has been suggested to be the driver of genetic changes in the *cytochrome b* gene in both Antarctic types B and C (Foote *et al.*, 2011b). A single non-synonymous amino acid substitution resulting in a directional change in amino acid polarity was estimated to have occurred under positive selection and was close to fixation in type C. A non-synonymous amino acid substitution at another site, which resulted in the opposite change in the polarity of the amino acid, was found to be fixed in type B and was also estimated to have occurred under positive selection (Foote *et al.*, 2011b). The changes are expected to have an impact on adenosine triphosphate (ATP) production and hence metabolic performance (Foote *et al.*, 2011b). Both these types, which are sister taxa, inhabit the Antarctic pack ice and the thermal challenges of this environment may be an important selection pressure. The substitution found in type C may be additionally linked to body size, as this is the smallest of the killer whales types described to date (Pitman *et al.*, 2007) (Fig. 1). Non-synonymous amino acid changes were found at the same or a neighbouring site, resulting in the same directional change in local polarity, and a signature of positive selection of similar magnitude in comparisons of the *cytochrome b* gene of the sperm whale *Physeter macrocephalus* with the dwarf and pygmy sperm whales *Kogia* spp., and the hippopotamus *Hippopotamus amphibius* with the pygmy hippopotamus *Choeropsis liberiensis* (A.D. Foote, unpublished data).

In summary, there is evidence for adaptive divergence among killer whale types due to natural selection in the North Pacific and Northeast Atlantic, but to date this has been limited to non-heritable plastic traits, which as noted above and in the following sections, could promote or constrain the progress towards ecological speciation. There is some evidence for adaptive divergence among Antarctic killer whale types of heritable traits.

Is there evidence for reproductive isolation?

In four decades of field-based studies in the Northeast Pacific, the transient and resident types have appeared to be completely socially isolated (Ford and Ellis, 1999; Ford *et al.*, 2000). In fact, transients have been observed to change course to avoid contact once calls of the resident type have been detected, and there have been a few observations of aggressive interactions between them (Morton, 1990; Ford and Ellis, 1999). Analysis of mitochondrial DNA and microsatellites detected no permanent dispersal and high genetic differentiation among North Pacific ecotypes (Hoelzel *et al.*, 1998, 2007; Barrett-Lennard and Ellis, 2001; Morin *et al.*, 2010; M.J. Ford *et al.*, 2011). Uncertainty remains over the degree of reproductive isolation, as some studies have reported estimates of low-level ongoing male-mediated gene flow between types (Hoelzel *et al.*, 2007; Pilot *et al.*, 2010) and incongruence between nuclear and mitochondrial DNA trees (Barrett-Lennard and Ellis, 2001; Pilot *et al.*, 2010); others have found no evidence for contemporary male-mediated gene flow (M.J. Ford *et al.*, 2011). Therefore, although there is some debate over whether it is complete or not, there does appear to be some degree of reproductive isolation between North Pacific killer whale ecotypes. Genetic differentiation, based on microsatellite genotypes, indicates there is also a degree of reproductive isolation between Antarctic types (Morin *et al.*, 2010). There are currently no data to test this with for the North Atlantic.

Is reproductive isolation associated with adaptations to ecology and therefore consistent with ecological speciation?

Comparing genetic differentiation within and between ecotypes

The review above shows that three of the key prerequisites for ecological speciation – ecological variation, adaptive phenotype variation, and reproductive isolation – are to some degree present in our study subject, the killer whale. However, to robustly infer ecological speciation, a causal mechanism linking these different components must be demonstrated. A key indicator of this link and therefore a predicted outcome of ecological speciation is that the level of differentiation should be greater among populations of different ecotypes than among populations of the same ecotype (Schluter, 2001). Studies comparing genetic differentiation among populations in the North Pacific have found there is typically greater genetic differentiation between ecotypes than within each ecotype (e.g. Barrett-Lennard and Ellis, 2001; Hoelzel *et al.*, 2007) (see Fig. 5). However, the differences may be partially explained by the time since divergence, as the degree of reproductive isolation is expected to correlate with time since divergence (see Coyne and Orr, 1989, 2004) (see Fig. 6). In comparisons of genetic differentiation between ecotypes, the variation that is not explained by time since divergence (see Fig. 6) is interesting, as it may give an indication of differences in the rate of evolution

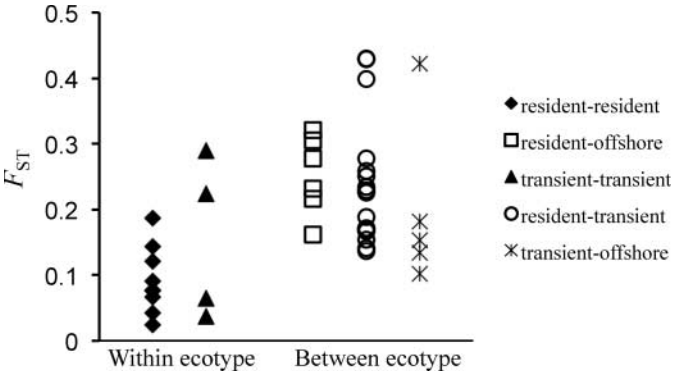

Fig. 5. Genetic differentiation between populations of North Pacific killer whales based on neutral nuclear DNA markers (microsatellites) using Weir and Cockerham's (1984) F_{ST} based on data in Hoelzel *et al.* (2007) and Barratt-Lennard and Ellis (2001).

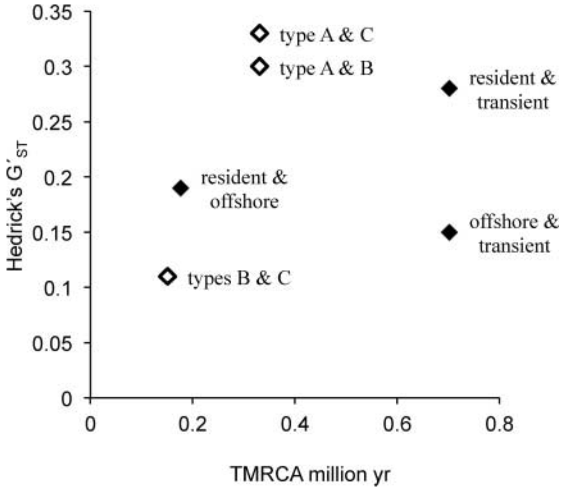

Fig. 6. A comparison of genetic differentiation between North Pacific ecotypes (solid diamonds) and between Antarctic ecotypes (open diamonds) based on neutral nuclear DNA markers (microsatellites) using Hedrick's (2005) G'_{ST}, plotted against time since divergence, based on data in Morin *et al.* (2010).

of reproductive isolation between different killer whale types. This would suggest that reproductive isolation may be more strongly promoted between the resident and the transient ecotypes than between the offshore and transient ecotypes in the North Pacific, and between types A and both types B and C than between type B and type C in the Antarctic (Fig. 6).

Post-mating isolation

Ecology-dependent adaptations could result in reproductive isolation through reducing immigrant or hybrid fitness or through assortative mating. As noted above, long-term studies of identified individuals have never observed permanent immigration into a population (Ford *et al.*, 2000). Although there has been hybridization between different killer whale types in captivity, it is not known if these are viable (see Riesch *et al.*, 2012), and does not allow a test of their fitness under natural conditions. One genetics study identified potential hybrids in the North Pacific (Pilot *et al.*, 2010), but no studies have investigated differences in fitness between these few individuals and non-hybrids. To date, no published reports have identified individuals with intermediate morphological traits of the Antarctic types (Pitman and Ensor, 2003), suggesting a low frequency of hybridization. Theoretically, any of the genetically heritable traits mentioned above, if under selection due to ecology, could reduce hybrid fitness. However, the plastic traits such as vocal rates and group size, for which the most convincing evidence of ecological selection exists, are thought to be culturally inherited, with predominantly vertical transmission within matrilineal groups (see Riesch *et al.*, 2012). Cultural inheritance of these behavioural differences might be expected to select against immigrants, especially if behavioural plasticity is limited at the age the immigrant individual disperses (Riesch *et al.*, 2012; see Thibert-Plante and Hendry, 2011). However, adaptive culturally inherited traits would not select against hybridization through male-mediated gene flow, as the offspring would still culturally inherit the adaptive phenotype suited to the ecology of its natal group (Hoelzel *et al.*, 2007).

Pre-mating isolation

Riesch *et al.* (2012) recently suggested a role for culturally transmitted traits, in particular stereotyped call repertoires, as cues for assortative mating in killer whales. These stereotyped call dialects are thought to be a product of cultural drift, social selection, and founding effects (Filatova *et al.*, 2012). If killer whale dialects are not under natural selection due to ecology, each dialect will be neutral with regard to its fitness in different ecological contexts. Assortative mating based on cultural traits that evolve solely through founding effects, social selection, and cultural drift would not be an example of ecological speciation (Price, 2008; Schluter, 2009).

Assortative mating would have to be based on ecology-dependent characteristics of killer whale call repertoires for such a mechanism to be classified as ecological speciation (for a reviews on this process, see Boughman, 2002; Slabbekoorn and Smith, 2002). For example, Foote and Nystuen (2008) found some correlations between the frequency characteristics of a selection of calls produced by each North Pacific ecotype and the hearing characteristics of their preferred prey and background noise of their preferred habitat. These putatively adaptive ecology-dependent characteristics were hypothesized to reduce detection by prey and enhance transmission characteristics (Foote and Nystuen, 2008). Assortative mating based on ecology-dependent characteristics of cultural traits such as killer whale calls or birdsong could also be based on a relationship between physical differences linked to ecology, which are then somehow reflected in the vocalizations. For example, beak size in Darwin's finches can be under natural selection due to preferred diet; beak size then influences song pitch, which influences mate choice and leads to assortative mating between ecologically divergent types (see De León *et al.*, this issue and references therein). Miller *et al.* (2007) found differences in the spectral characteristics – the distribution of energy across harmonics – in comparisons between males and females, which given the marked sexual size dimorphism in killer whales, they attributed to differences in body size. In a recent study, M.J. Ford *et al.* (2011) found that within a killer whale population, only the largest full-grown adult males had sired offspring. Therefore, these vocal cues based on physical size could potentially act in mate choice. Female mate choice based on body size within a population would be an example of sexual selection rather than ecological speciation. However, if the variation in body size exhibited among killer whale types results from natural selection, and this in turn causes differences in mate choice cues such as the spectral characteristics of vocalizations, then this could potentially lead to the evolution of ecology-dependent divergence in sexual signalling and mate choice that would be considered an example of ecological speciation (Schluter, 2000).

At present, however, these ideas remain speculative and the hypotheses untested. To date there is no conclusive evidence other than descriptive correlations that call dialects or any other phenotype traits are a causal link to mate choice or low hybrid fitness and underlie reproductive isolation between killer whale ecotypes. So the case for robustly determining if ecological speciation is the mechanism driving evolutionary divergence in killer whales falters at conclusively finding the causal links between ecological variation, phenotype variation, and reproductive isolation. I suspect that this is probably the case for many studies striving to determine if ecological speciation is operating in non-model organisms. Without the ability to perform experimental investigations such as translocations and laboratory experiments on mate choice, studies remain purely descriptive, and without large numbers of taxon pairs with clear parallel divergences in ecology and adaptive traits to compare between, such descriptive studies lack power.

ALTERNATIVE MECHANISMS TO ECOLOGICAL SPECIATION

Given that the case for ecological speciation in the killer whale is inconclusive, what alternative mechanisms should be considered that could underlie the observed population structure? Temporary bottlenecks, such as caused by founder events or periods in glacial refugia, can cause a substantial and prolonged loss of genetic and cultural diversity (Mayr, 1942; Templeton, 1980; Baker and Jenkins, 1987; Powell *et al.*, 2009; Atkinson, 2011). Smaller populations will diverge more rapidly due to genetic and cultural drift, which can promote phenotypic divergence, genetic divergence, and ultimately speciation (e.g. Mayr, 1942; Templeton, 1980; Powell *et al.*, 2009; Atkinson, 2011; Kolbe *et al.*, 2012).

A mechanism along these lines was suggested as the underlying basis for population structure in killer whales (Hoelzel *et al.*, 2007). There is low genetic variation both locally and globally in killer whales, potentially linked to a historic bottleneck and local founding events (Hoelzel *et al.*, 2002; Morin *et al.*, 2010; Foote *et al.*, 2011a). Estimates of effective migrants that moved from the Atlantic to the Pacific and founded the resident and offshore ecotypes were very small, perhaps even a single female (Foote *et al.*, 2011a). Additionally, all individuals within local populations of each ecotype often have single fixed haplotypes, suggesting additional localized founding events (Barrett-Lennard and Ellis, 2001; Hoelzel *et al.*, 2007; Foote *et al.*, 2011c). Hoelzel *et al.* (2007) argued that social philopatry within regional populations would explain the fixation of mtDNA haplotype, and suggested that the level of male-mediated gene flow during temporary interactions between populations would be dependent upon the probability of such interactions occurring. They proposed that differences in habitat usage would reduce the number of temporary interactions between different ecotypes relative to the number of interactions between populations of the same ecotype. This explanation would be consistent with the difference in the level of genetic differentiation between populations in comparisons between and within ecotypes (Hoelzel *et al.*, 2007).

Of course, these ideas are not mutually exclusive with ecological speciation due to natural selection. A recent experimental study on Anolis lizards found evidence for a role of both natural selection and founder effects in evolutionary divergence (e.g. Kolbe *et al.*, 2012). Natural selection led to directional evolution of adaptive phenotypes in several independent locations; founder effects led to additional morphological and genetic variation among locations (e.g. Kolbe *et al.*, 2012). There are other possible mechanisms underlying speciation, such as Dobzhansky-Muller incompatibilities between mutations accumulated in different populations, sexual selection, and polyploidy, but I will not consider these in detail here. Suffice to say, there are other possible scenarios other than divergence due to ecological speciation that could have led to the observed pattern of ecologically and phenotypically divergent, reproductively isolated killer whale types.

A BOTTOM-UP APPROACH TO STUDYING ECOLOGICAL SPECIATION IN NON-MODEL ORGANISMS

Population genomic studies using genome-wide scan data from multiple individuals are now becoming more accessible due to the advent of highly parallel, high-throughput sequencing and newly developed restriction assay digest (RAD) methods, allowing the cost-effective sequencing of reduced representation libraries (RRLs) of the genome (e.g. Baird *et al.*, 2008; Elshire *et al.*, 2011). These protocols are useful in investigations of ecological speciation not only because of the sheer magnitude of genetic data they produce, but because they can include loci linked to adaptive traits and therefore evolving under natural selection (e.g. Hohenlohe *et al.*, 2010; Jones *et al.*, 2012). Studies of killer whale population structure have used microsatellite loci, thought to be neutral with respect to natural selection. These markers are commonly used in studies on ecological speciation (e.g. Huber *et al.*, 2007; Berner *et al.*, 2009). However, neutral loci can fail to detect the effects of natural selection on loci linked to adaptive traits (Thibert-Plante and Hendry, 2010). The assumption made when using neutral genetic markers to investigate the progress towards ecological speciation is that selection on adaptive markers will cause a generalized barrier to gene flow that will then be detectable using non-linked neutral loci (Thibert-Plante and Hendry, 2010). However, under many conditions detection of this barrier is unlikely to occur. For example, in scenarios with low levels of migration between small populations, as in the case of North Pacific killer whales (Hoelzel *et al.*, 2007), genetic differentiation will build up between all populations caused by divergence due to drift, which will mask any influence of a generalized barrier to gene flow caused by natural selection (Thibert-Plante and Hendry, 2010). There can also be sharing of neutral loci unlinked to selected markers due to gene flow between ecotypes, but post-zygotic barriers (e.g. low hybrid fitness) to the gene flow of adaptive alleles at loci under selection between ecotypes. This can result in a pattern of homogenization of allele sharing at neutral loci across ecological barriers, but differentiation based on ecologically selected alleles at loci linked to adaptive traits (e.g. Jones *et al.*, 2012).

Genome-wide scans used to detect loci under positive selection and loci/trait mapping to identify which phenotypic traits these are linked to, can provide insights into whether parallel evolution of traits under natural selection is occurring independently in different populations (e.g. Deagle *et al.*, 2012; Jones *et al.*, 2012; Roesti *et al.*, 2012). In non-model organisms such as killer whales this is useful, as it can potentially increase the number of traits identified as being under selection, and allows a standardized quantitative comparison for measuring the strength of selection across all identified adaptive loci/traits and between different populations (Linnen and Hoekstra, 2009). This can increase the power of comparative studies of non-model organisms such as the killer whale, which may have few taxon pairs to compare among for signs of parallel divergence, i.e. a high number of traits/loci in few taxon pairs approach, versus few traits in larger numbers of taxon pairs approach used in many classic studies of model organisms. However, it may be that there are a number of combinations of genotypic variation that could arrive at the same adaptive phenotypic outcome (Elmer and Meyer, 2011), which may hamper identifying parallel evolution from genome-wide scan data.

A bottom-up approach to robustly determining if ecological speciation is the underlying mechanism of evolution divergence of a non-model organism would therefore address the key questions in the following way:

1. *The identification of ecological differences between speciating taxa*. Samples would be binned based on ecological differences *a priori* to the genetic investigation.

2. *The identification of adaptive divergence due to natural selection of phenotypic traits associated with these ecological differences*. Adaptive divergence would be identified by outlier loci within genes exhibiting particularly high differentiation compared with the majority of neutral loci scanned across the genome in pairwise comparisons between ecotypes. Higher differentiation at a locus would result from alternative adaptive alleles reaching fixation in a different ecotype due to natural selection. This is expected to be most effective in scenarios where there is ongoing gene flow to reduce differentiation across the neutral regions of the genome.

3. *Determining that these diverging taxa are reproductively isolated*. The degree of sharing of alleles of both neutral and adaptive markers would indicate the degree of reproductive isolation.

4. *The identification of a causal link between the adaptive traits under natural selection and reproductive isolation*. Outlier loci indicate that alleles at these loci are being exchanged between ecotypes at a lower rate than the rest of the genome. Therefore the adaptive mutations in these genes must also be linked to reproduction isolation. This could either be due to mate choice based on the phenotypic expression of adaptive alleles, or low fitness of hybrids lacking the adaptive allele for their ecological niche. The exact mechanism linking the adaptive alleles and reproductive isolation may not be identified by genome-wide scans alone, but this approach can then be followed up by genetic mapping to link these alleles to phenotypic traits and then more traditional approaches can be applied to investigate the role of these traits in reproductive isolation.

Caveats to the use of genome-wide scans to investigate speciation processes

The application of genome-wide scans to better understand speciation is still in its infancy and as such some of the limitations of this approach are only just starting to be better appreciated. Ultimately, this method relies upon the ability to discriminate between loci under selection and those evolving under neutrality. There are a number of processes that can reduce the resolution of identifying genes under selection from genome-wide scans. For example, it is important to have some previous understanding of the geographic context of speciation and the level of gene flow in the study system; since at one extreme, generalized barriers to gene flow such as allopatric divergences would lead to differentiation across the whole genome and mask any regions under selection. Even in the face of gene flow, the ability to detect adaptive changes will depend upon the strength of selection compared with the rate of neutral gene flow, and loci evolving under weak selection may not be detected. Neutral regions adjacent to loci under selection are also expected to show high differentiation due to linkage. The size of these outlier regions, or ‘genomic islands’, surrounding selected loci will depend upon the recombination rate and may therefore vary widely. These factors can make it difficult to identify the particular loci under selection and low-density genome-wide scans run the risk of misidentifying neutral regions flanking outlier loci as being adaptive and evolving under selection (see Roesti *et al.*, 2012). A recent collection of papers on patterns and processes of genomic divergence during speciation edited by Nosil and Feder (2012) provides an excellent overview of these issues and caveats.

CONCLUSIONS

The difficulty of robustly determining if ecological speciation underlies the pattern of evolutionary variation observed in some non-model organisms is highlighted by this case study on the killer whale. However, there is much promise in new molecular approaches that initially identify selection on the genotype rather than the phenotype. Model biological systems that have provided robust evidence for ecological speciation are likely to remain the most useful systems for furthering our understanding of ecological speciation, e.g. investigating the role of individuality in initiating speciation (see De León *et al.*, this issue), or variables that determine the rate of speciation (Grant and Grant, 2002; Losos *et al.*, 2006; Taylor *et al.*, 2006; Berner *et al.*, 2009). However, it is important that we strive to move beyond merely describing differentiation of ecological, morphological, and neutral genetic markers in non-model organisms, and try to understand the underlying processes determining diversification (see Crandall *et al.*, 2000). Understanding the process can help inform management decisions such as selection of individuals for translocations and reintroduction programmes and a more qualified determination of taxonomic status.

ACKNOWLEDGEMENTS

I would like to thank Andrew Hendry for his helpful advice during the preparation of this manuscript, and I would additionally like to thank Bob Pitman, John Durban, Phil Morin, Rus Hoelzel, and others in the killer whale research community for lively discussions on the topic of evolution and speciation in killer whales over the years. I'm sure this is far from being the last word on the matter. Financial support for this work came from an EU Marie Curie Actions ITN Fellowship 'KWAF10' grant.

REFERENCES

Andrews, R.D., Pitman, R.L. and Ballance, L.T. 2008. Satellite tracking reveals distinct movement patterns for Type B and Type C killer whales in the southern Ross Sea, Antarctica. *Polar Biol.*, **31**: 1461–1468.

Atkinson, Q.D. 2011. Phonemic diversity supports a serial founder effect model of language expansion from Africa. *Science*, **332**: 346–349.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A. *et al.* 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE*, **3**(10): e3376.

Baird, R.W. and Dill, L.M., 1996. Ecological and social determinants of group size in transient killer whales. *Behav. Ecol.*, **7**: 408–416.

Baker, A.J. and Jenkins, P.F. 1987. Founder effect and cultural evolution of songs in an isolated population of chaffinches, *Fringilla coelebs*, in the Chatham Islands. *Anim. Behav.*, **35**: 1793–1803.

Barraclough, T.G. and Nee, S. 2001. Phylogenetics and speciation. *Trends Ecol. Evol.*, **16**: 391–399.

Barrett-Lennard, L.G. and Ellis, G.M. 2001. *Population structure and genetic variability in Northeastern-Pacific killer whales: Towards an assessment of population viability*. Canadian

Science Advisory Secretariat Research Document, 2001/65, Fisheries and Oceans Canada, Nanaimo, British Columbia, Canada.

Barrett-Lennard, L.G., Ford, J.K.B. and Heise, K.A. 1996. The mixed blessing of echolocation: differences in sonar use by fish-eating and mammal-eating killer whales. *Anim. Behav.*, **51**: 553–565.

Beck, S., Kuningas, S., Esteban-Pavo, R. and Foote, A.D. 2012. The influence of ecology on sociality in the killer whale (*Orcinus orca*). *Behav. Ecol.*, **23**: 246–253.

Berner, D., Grandchamp, A.-C. and Hendry, A.P. 2009. Variable progress toward ecological speciation in parapatry: stickleback across eight lake–stream transitions. *Evolution*, **63**: 1740–1753.

Boughman, J.W. 2002. How sensory drive can promote speciation. *Trends Ecol. Evol.*, **17**: 571–577.

Burdin, A., Hoyt, E., Sato, H. and Filatova, O. 2005. *Resident and transient-type killer whales*, Orcinus orca*, in Southeast Kamchatka, Russia*. Report to Scientific Committee of the International Whaling Commission, Anchorage. SC/56/SM15.

Coyne, J.A. and Orr, H.A. 1989. Patterns of speciation in *Drosophila. Evolution*, **43**: 362–381.

Coyne, J.A. and Orr, H.A. 2004. *Speciation*. Sunderland, MA: Sinauer Associates.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M. and Wayne, R.K. 2000. Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.*, **15**: 290–295.

Crispo, E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. *J. Evol. Biol.*, **21**: 1460–1469.

Dahlheim, M.E., Schulman-Janiger, A., Black, N., Ternullo, R., Ellifrit, D. and Balcomb, K.C., III. 2008. Eastern temperate North Pacific offshore killer whales (*Orcinus orca*): occurrence, movements, and insights into feeding ecology. *Mar. Mamm. Sci.*, **24**: 719–729.

Darwin, C. 1859. *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. London: John Murray.

Deagle, B.E., Jones, F.C., Chan, Y.F., Absher, D.M., Kingsley, D.M. and Reimchen, T.E. 2012. Population genomics of parallel phenotypic evolution in stickleback across stream–lake ecological transitions. *Proc. R. Soc. Lond. B*, **279**: 1277–1286.

Deecke, V.B., Slater, P.J.B. and Ford, J.K.B. 2002. Selective habituation shapes acoustic predator recognition in harbour seals. *Nature*, **420**: 171–173.

Deecke, V.B., Ford, J.K.B. and Slater, P.J.B. 2005. The vocal behaviour of mammal-eating killer whales: communicating with costly calls. *Anim. Behav.*, **69**: 395–405.

Deecke, V.B., Nykänen, M., Foote, A.D. and Janik, V.M. 2011. Vocal behavior and feeding ecology of killer whales (*Orcinus orca*) around Shetland, UK. *Aquat. Biol.*, **13**: 79–88.

De León, L.F., Rolshausen, G., Bermingham, E., Podos, J. and Hendry, A.P. 2012. Individual specialization and the seeds of adaptive radiation in Darwin's finches. *Evol. Ecol. Res.*, **14**: 000–000.

Dobzhansky, T. 1937. *Genetics and the Origin of Species*. New York: Columbia University Press.

Durban, J.W. and Pitman, R.L. 2012. Antarctic killer whales make rapid, round-trip movements to subtropical waters: evidence for physiological maintenance migrations? *Biol. Lett.*, **8**: 274–277.

Elmer, K.R. and Meyer, A. 2011. Adaptation in the age of ecological genomics: insights from parallelism and convergence. *Trends Ecol. Evol.*, **26**: 298–306.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S. *et al.* 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, **6**(5): e19379.

Fearnbach, H., Durban, J.W., Ellifrit, D.K. and Balcomb, K.C., III 2011. Size and long-term growth trends of endangered fish-eating killer whales. *Endangered Species Res.*, **13**: 173–180.

Filatova, O.A., Deecke, V.B., Ford, J.K.B., Matkin, C.O., Barrett-Lennard, L.G., Guzeev, M.A. *et al.* 2012. Call diversity in the North Pacific killer whale populations: implications for dialect evolution and population history. *Anim. Behav.*, **83**: 595–603.

Foote, A.D. and Nystuen, J.A. 2008. Variation in call pitch among killer whale ecotypes. *J. Acoust. Soc. Am.*, **123**: 1747–1752.

Foote, A.D., Newton, J., Piertney, S.B., Willerslev, E. and Gilbert, M.T.P. 2009. Ecological, morphological and genetic divergence of sympatric North Atlantic killer whale populations. *Mol. Ecol.*, **18***:* 5207–5217.

Foote, A.D., Morin, P.A., Durban, J.W., Willerslev, E., Orlando, L. and Gilbert, M.T.P. 2011a. Out of the Pacific and back again: the matrilineal history of Pacific killer whale ecotypes. *PLoS ONE*, **6**(9): e24980.

Foote, A.D., Morin, P.A., Durban, J.W., Pitman, R.L., Wade, P., Willerslev, E. *et al.* 2011b. Positive selection on the killer whale mitogenome. *Biol. Lett.*, **7**: 116–118.

Foote, A.D., Vilstrup, J.T., De Stephanis, R., Verborgh, P., Abel Nielsen, S.C., Deaville, R. *et al.* 2011c. Genetic differentiation among North Atlantic killer whale populations. *Mol. Ecol.*, **20**: 629–641.

Ford, J.K.B. and Ellis G.M. 1999. *Transients: Mammal-Hunting Killer Whales of British Columbia, Washington, and Southeastern Alaska*. Vancouver, BC: UBC Press.

Ford, J.K.B., Ellis, G.M., Barrett-Lennard, L.G., Morton, A.B., Palm, R.S. and Balcomb, K.C. 1998. Dietary specialization in two sympatric populations of killer whale (*Orcinus orca*) in coastal British Columbia and adjacent waters. *Can. J. Zool.*, **76**: 1456–1471.

Ford, J.K.B., Ellis, G.M. and Balcomb, K.C. 2000. *Killer Whales, The Natural History and Genealogy of* Orcinus orca *in British Columbia and Washington*. Vancouver, BC: UBC Press.

Ford, J.K.B., Ellis, G.M., Matkin, C.O., Wetklo, M.H., Barrett-Lennard, L.G. and Withler, R.E. 2011. Shark predation and tooth wear in a population of northeastern Pacific killer whales. *Aquat. Biol.*, **11**: 213–224.

Ford, M.J., Hanson, M.B., Hempelmann, J.A., Ayres, K.L., Emmons, C.K., Schorr, G.S. *et al.* 2011. Inferred paternity and male reproductive success in a killer whale (*Orcinus orca*) population. *J. Hered.*, **102**: 537–553.

Funk, D.J., Nosil, P. and Etges, W.J. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proc. Natl. Acad. Sci. USA*, **103**: 3209–3213.

Grant, P.R. and Grant, B.R. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science*, **296**: 707–711.

Grant, P.R. and Grant, B.R. 2008. *How and Why Species Multiply: The Radiation of Darwin's Finches*. Princeton, NJ: Princeton University Press.

Hedrick, P.W. 2005. A standardized genetic differentiation measure. *Evolution*, **59**: 1633–1638.

Hendry, A.P. 2009a. Ecological speciation! Or the lack thereof? *Can. J. Fish. Aquat. Sci.*, **66**: 1383–1398.

Hendry, A.P. 2009b. Evolutionary biology: speciation. *Nature*, **458**: 162–164.

Herman, D.P., Burrows, D.G., Wade, P.R., Durban, J.W., Matkin, C.O., LeDuc, R.G. *et al.* 2005. Feeding ecology of eastern North Pacific killer whales *Orcinus orca* from fatty acid, stable isotope and organochlorine analyses of blubber biopsies. *Mar. Ecol. Progr. Ser.*, **302**: 275–291.

Hoelzel, A.R., Dahlheim, M.E. and Stern, S.J. 1998. Low genetic variation among killer whales in the eastern North Pacific, and genetic differentiation between foraging specialists. *J. Hered.*, **89**: 121–128.

Hoelzel, A.R., Natoli, A., Dahlheim, M.E., Olavarria, C., Baird, R.W. and Black, N.A. 2002. Low worldwide genetic diversity in the killer whale (*Orcinus orca*): implications for demographic history. *Proc. R. Soc. Lond. B*, **269**: 1467–1475.

Hoelzel, A.R., Hey, J., Dahlheim, M.E., Nicholson, C., Burkanov, V. and Black, N. 2007. Evolution of population structure in a highly social top predator, the killer whale. *Mol. Biol. Evol.*, **24**: 1407–1415.

Hohenlohe, P.A., Bassham, S., Etter, P.D., Stiffler, N., Johnson, E.A. and Cresko, W.A. 2010. Population genomics of parallel adaptation in threespine stickleback using RAD sequenced tags. *PLoS Genet.*, **6**(2): e1000862.

Huber, S.K., De León, L.F., Hendry, A.P., Berminham, E. and Podos, J. 2007. Reproductive isolation of sympatric morphs in a population of Darwin's finches. *Proc. R. Soc. Lond. B*, **274**: 1709–1714.

Jones, F.J., Yinguang, F.C., Schmutz, J., Grimwood, J., Brady, S.D., Southwick, A.M. *et al.* 2012. A genome-wide SNP genotyping array reveals patterns of global and repeated species-pair divergence in sticklebacks. *Curr. Biol.*, **22**: 83–90.

Kolbe, J.J., Leal, M., Schoener, T.W., Spiller, D.A. and Losos, J.B. 2012. Founder effects persist despite adaptive differentiation: a field experiment with lizards. *Science*, **335**: 1086–1089.

Linnen, C.R. and Hoekstra, H.E. 2009. Measuring natural selection on genotypes and phenotypes in the wild. *Cold Spring Harb. Symp. Quant. Biol.*, **74**: 155–168.

Losos, J.B. 2009. *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. Berkeley, CA: University of California Press.

Losos, J.B., Schoener, T.W., Langerhans, R.B. and Spiller, D.A. 2006. Rapid temporal reversal in predator-driven natural selection. *Science*, **314**(5802): 1111.

Mayr, E. 1942. *Systematics and the Origin of Species*. New York: Columbia University Press.

McKinnon, J.S.M. and Rundle, H.D. 2002. Speciation in nature: the threespine stickleback model systems. *Trends Ecol. Evol.*, **17**: 480–488.

McKinnon, J.S., Mori, S., Blackman, B.K., David, L., Kingsley, D.M., Jamieson, L. *et al.* 2004. Evidence for ecology's role in speciation. *Nature*, **429**: 294–298.

Miller, P.J.O., Samarra, F.I.P. and Perthuison, A.D. 2007. Caller sex and orientation influence spectral characteristics of 'two-voice' stereotyped calls produced by free-ranging killer whales. *J. Acoust. Soc. Am.*, **121**: 3932–3937.

Morin, P.A., Archer, F.I., Foote, A.D., Vilstrup, J., Allen, E.E., Wade, P. *et al.* 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Res.*, **20**: 908–916.

Morton, A.B. 1990. A quantitative comparison of the behaviour of resident and transient forms of killer whales off the central British Columbia coast. *Rep. Int. Whaling Commn.*, special issue **12**: 245–248.

Nagel, L. and Schluter, D. 1998. Body size, natural selection, and speciation in sticklebacks. *Evolution*, **52**: 209–218.

Nosil, P. and Feder, J.L. 2012. Patterns and processes of genomic divergence during speciation. *Phil. Trans. R. Soc. Lond. B*, **367**: 332–474.

Nosil, P., Harmon, L.J. and Seehausen, O. 2009. Ecological explanations for (incomplete) speciation. *Trends Ecol. Evol.*, **24**: 145–156.

Orr, M.R. and Smith, T.B. 1998. Ecology and speciation. *Trends Ecol. Evol.*, **13**: 502–506.

Peichel, C.L., Nereng, K.S., Ohgi, K.A., Cole, B.L.E., Colosimo, P.F., Buerkle, C.A. *et al.* 2001. The genetic architecture of divergence between threespine stickleback species. *Nature*, **414**: 901–905.

Pilot, M., Dahlheim, M.E. and Hoelzel, A.R. 2010. Social cohesion among kin, gene flow without dispersal and the evolution of population structure in the killer whale (*Orcinus orca*). *J. Evol. Biol.*, **23**: 20–31.

Pitman, R.L. and Durban, J.W. 2010. Killer whale predation on penguins in Antarctica. *Polar Biol.*, **33**: 1589–1594.

Pitman, R.L. and Durban, J.W. 2012. Cooperative hunting behavior, prey selectivity and prey handling by pack ice killer whales (*Orcinus orca*), type B, in Antarctic Peninsula waters. *Mar. Mammal Sci.*, **28**: 16–36.

Pitman, R.L. and Ensor, P. 2003. Three forms of killer whales (*Orcinus orca*) in Antarctic waters. *J. Cetacean Res. Manage.*, **5**: 131–139.

Pitman, R.L., Perryman, W.L., LeRoi, D. and Eilers, E. 2007. A dwarf form of killer whale in Antarctica. *J. Mammal.*, **88**: 43–48.

Pitman, R.L., Durban, J.W., Greenfelder, M., Guinet, C., Jorgensen, M., Olson, P. *et al.* 2011. Observations of a distinctive morphotype of killer whale (*Orcinus orca*), type D, from subantarctic waters. *Polar Biol.*, **34**: 303–306.

Powell, A., Shennan, S. and Thomas, M.G. 2009. Late Pleistocene demography and the appearance of modern human behavior. *Science*, **324**: 1298–1301.

Price, T. 2008. *Speciation in Birds*. Greenwood Village, CO: Roberts & Co.

Riesch, R.R., Barrett-Lennard, L.G., Ellis, G.M., Ford, J.K.B. and Deecke, V.B. 2012. Cultural traditions and the evolution of reproductive isolation: ecological speciation in killer whales? *Biol. J. Linn. Soc.*, **106**: 1–17.

Roesti, M., Hendry, A.P., Sazburger, W. and Berner, D. 2012. Genome divergence during evolutionary diversification as revealed in replicate lake–stream stickleback population pairs. *Mol. Ecol.*, **21**: 2852–2862.

Rundle, H.D. and Nosil, P. 2005. Ecological speciation. *Ecol. Lett.*, **8**: 336–352.

Saulitis, E.L., Matkin, C.O., Barrett-Lennard, L.G., Heise, K.A. and Ellis, G.M. 2000. Foraging strategies of sympatric killer whale (*Orcinus orca*) populations in Prince William Sound, Alaska. *Mar. Mammal Sci.*, **16**: 94–109.

Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford: Oxford University Press.

Schluter, D. 2001. Ecology and origin of the species. *Trends Ecol. Evol.*, **16**: 372–380.

Schluter, D. 2009. Evidence for ecological speciation and its alternative. *Science*, **323**: 737–741.

Schluter, D. and Nagel, L.M. 1995. Parallel speciation by natural selection. *Am. Nat.*, **146**: 292–301.

Slabbekoorn, H. and Smith, T.B. 2002. Bird song, ecology, and speciation. *Phil. Trans. R. Soc. Lond. B*, **357**: 493–503.

Stenersen, J. and Similä, T. 2004. *Norwegian Killer Whales*. Henningsvær, Norway: Tringa Forlag.

Taylor, E.B., Boughman, J.W., Groenenboom, M., Sniatynski, M., Schluter, D. and Gow, J.L. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Mol. Ecol.*, **15**: 343–355.

Templeton, A.R. 1980. The theory of speciation via the founder principle. *Genetics*, **94**: 1011–1038.

Thibert-Plante, X. and Hendry, A.P. 2010. When can ecological speciation be detected with neutral loci? *Mol. Ecol.*, **19**: 2301–2314.

Thibert-Plante, X. and Hendry, A.P. 2011. The consequences of phenotypic plasticity for ecological speciation. *J. Evol. Biol.*, **24**: 326–342.

Weir, B.S. and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**: 1358–1370.

Zerbini, A.N., Waite, J.M., Durban, J.W., LeDuc, R., Dahlheim, M.E. and Wade, P.R. 2007. Estimating abundance of killer whales in the nearshore waters of the Gulf of Alaska and Aleutian Islands using line-transect sampling. *Mar. Biol.*, **150**: 1033–1045.