Fluctuating asymmetry in the saddle patch shape of the Pacific Ocean killer whale (*Orcinus orca*) populations

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The killer whale (*Orcinus orca*) is a top predator and one of the most contaminated marine mammal species in the world. Due to different prey preferences and life styles, killer whale populations accumulate persistent pollutants differently, and therefore are exposed differently to this stress. Stress may express itself in a population as an increase in the relative number of individuals with asymmetric presentation of a trait that is normally symmetrical. This phenomenon is called fluctuating asymmetry. There are many environmental and genetic factors that can cause fluctuating asymmetry. We have used the symmetry of the killer whale’s saddle patch pattern behind the dorsal fin as an indicator of fluctuating asymmetry in six Pacific Ocean populations. The southern resident killer whale population seems to be remarkably more asymmetrical than the other studied populations. Although many possible environmental factors could cause asymmetry, we suggest that small population size, development of reproductively isolated ecotypes and possible inbreeding as genetic factors are causing asymmetry in the southern resident population.

Introduction

When normally bilaterally symmetric individuals are not able to undergo identical development on both sides of their body, they develop fluctuating asymmetry. Fluctuating asymmetry represents a measure of the sensitivity of development to environmental and genetic stress (Møller & Swaddle 1997). Environmental factors that reduce developmental stability and cause fluctuating asymmetry can be chemical pollution, unusual temperatures, parasites, deficient food sources, and even loud sounds (Møller 1996, Møller & Swaddle 1997). Genetic effects such as high levels of inbreeding, extreme homozygosity, hybridization, novel mutations, and periods of intense directional selection have been shown to reduce the ability of the organism to buffer its developmental pathways against the reproduction of random errors (Møller & Swaddle 1997).

There has been wide discussion and argument about developmental instability and fluctuating asymmetry as a fitness indicator. The developmental processes that lead to asymmetry
are still debated and the relative contributions of the different sources of variation are not known (Gangestad & Thornhill 2003). Results of studies measuring developmental instability using fluctuating asymmetry have been heterogeneous (Van Dongen 2006).

Fluctuating asymmetry of striped dolphins (Stenella coeruleoalba) that died during an epizootic in the Mediterranean was compared with that of the population prior to and after the epizootic. Significantly higher levels of fluctuating asymmetry were found in those individuals that died (Pertoldi et al. 2000). Baltic grey seal (Halichoerus grypus) and ringed seal (Pusa hispida) skull characters were more asymmetric during the time when organochlorine levels were high as compared with those before and after that (Zakharov et al. 1997). Dramatic change in developmental stability was also found in Baltic grey seals during the period of heavy pollution (Zakharov & Yablokov 1990). Fluctuating asymmetry has been reported in deer and antelope antlers (Arcese 1994, Folstad et al. 1996, Markusson & Folstad 1997), in several traits of birds (Manning & Hartley 1991, Swaddle 1996), and in many skeletal features in various species (Söderman et al. 2007), mostly in traits that affect individual appearance, display and behavior.

Killer whales around the world have a gray saddle-shaped pattern on both sides of the back behind the dorsal fin (Fig. 1). There are three to five main variants of the saddle-patch shape found around the world (Evans et al. 1982, Baird & Stacey 1988, Ford et al. 1994, Mäkeläinen 1999). Normally this saddle-patch pattern is symmetrical between sides, although some asymmetry may occur. In a sample of 52 individuals of the New Zealand killer whale population, 49 had symmetrical and three had asymmetrical saddle patches (Mäkeläinen 1999).

Killer whale calves are born with the main black and white color pattern (Fig. 1) as seen in adult individuals, except that the white pattern is somewhat yellowish or reddish. The saddle-patch pattern shows differently in young individ-
uals, depending on individual growth rates, skin regeneration, water conditions and algal blooms. It becomes consistently apparent after about two years of age. Once the pattern has formed it does not change (Bigg 1982). This developing color pattern can reflect the state of health of an individual due to environmental and genetic stress factors during the critical first years. Many of the persistent pollutants are accumulated in the adipose tissues and transported to a calf during lactation. Marine mammal males are reported to become increasingly contaminated as they grow older, while females off-load contaminants to their offspring during pregnancy and lactation (Borrell et al. 1995, Ross et al. 2000).

Fluctuating asymmetry in the saddle-patch pattern was studied in six Pacific Ocean killer whale populations (Fig. 2): (1) northern resident, (2) southern resident, (3) west coast transient, (4) Californian transient, (5) offshore, and (6) Kamchatkan. Some of these populations have partly overlapping ranges and some of them are reproductively isolated (Baird & Stacey 1988, Ford et al. 1994, Barrett-Lennard 2000). Besides differences in social organization and foraging ecology, residents, transients and offshore killer whales also differ in their dorsal fin- and saddle-patch shape (Baird & Stacey 1988, Ford et al. 2000).

Both resident killer whales ecotypes live their lives in their natal pods and eat mainly salmonid fish. Marine-mammal eating transient killer whales are divided into two populations: the Californian transients and the west-coast transients. Transient killer whales have a more flexible social organization than resident ones; they disperse from their natal groups and associate more freely. The fifth population, which is the least known, comprises fish-eating offshore killer whales. They are encountered in large groups and are found mostly far away from coastal waters between Alaska and California. The sixth population, the Kamchatkan killer whales, occur in distant Russian waters. All Kamchatkan killer whales in this study belong to the Kamchatkan resident type.

In this paper, we present a method for measuring the degree of symmetry in the saddle patch shape. Using this information, we analyzed differences in the degree of asymmetry in two of the Pacific Ocean killer whale populations. All six populations were also compared based on visual analyses.

**Material and methods**

Two methods were used to study asymmetry. First, all individuals of the six Pacific populations, for which photos of both sides were available, were compared visually. Second, a way to measure the asymmetry using Adobe Photoshop Elements was developed, and two of the populations were analyzed using this method (southern residents and Californian transients). For both methods, we used good left- and right-side photographs of each whale’s saddle patch and dorsal fin. Killer whales’ left sides are normally photographed for individual identification around the world. Smaller populations are also photographed regularly from the right side, other populations only occasionally.

**Method 1**

All saddle patches were classified into five variants by one person following the practices in...
the study of geographic variation in killer whale saddle patch pattern (Baird & Stacey 1988, Mäkeläinen 1999). Individuals that died or were missing for a while were also included in this study. Most of the variation in the saddle-patch shape is found in the front and upper part of the patch close to the fin, and this is the area on which we focused. The sides were compared and we noted if they were symmetrical, asymmetrical, or if there was only a small degree of asymmetry. The strength of the visual comparison was that even small asymmetries were detected. The differences in the distribution of individuals into asymmetry classes (symmetric, little asymmetric, asymmetric) were analyzed using a \( \chi^2 \)-test. Deviations from the expected distribution were analyzed by counting adjusted residuals. Deviations larger than ±1.96 indicate statistically significantly larger or smaller numbers of individuals in a particular asymmetry class.

**Method 2**

Saddle patch measurements were made using Adobe PhotoShop Elements. First, the left-side photograph was colored blue and the right-side red (shown as different shades of grey in Fig. 3b). This was done by enhancing the color hue/saturation to 220/50 and light to 0 for the blue image, and hue/saturation 0/100 and light to 50 for the red image. The right-side photograph was flipped horizontally to a mirror image. These differently colored photographs were super-
imposed, and the flipped right-side photo was resized to match the size of the left side. After that, the photos were separated. Saddle patches were outlined with a polygonal tool, cut from both photographs, pasted onto new layers (Fig. 3c), and superimposed (Fig. 3d). The lowest tips of both patches were cut along the same line and deleted. This way we removed different water lines and waves. Also the ends of the long stripes that continued towards the tail were deleted from the images, to minimize the effects of different positions in the pictures. By eliminating the tips and stripes, we identified the essential saddle-patch area were the pattern variation was found. Moreover, for both patches (left and right) the area was equal in size. If the photos of the left and right patches were taken from an individual at very different ages and were not comparable, such individuals were excluded.

When both patches were measured individually, the measured areas were chosen with a magic wand tool. After superimposing the patches, two areas were identified: (1) total area of patches, and (2) area where the patches do not overlap. Subsequently, the identified areas were selected with the software’s selection tool, and the numbers of pixels for the selected areas were recorded from the image histogram. The degree of asymmetry was expressed as a pixel ratio of non-overlapping area to total area. The degree of asymmetry of symmetrical patches equals 0. The amount of variation between populations was compared using coefficients of variation (CV, %).

Some individuals had saddle patches with blurred parts or outlines, hence finding the outlines was harder. However, in many cases, it was still possible to define the border between black and gray coloration. In these kinds of difficult cases visual comparison of the patches had its advantages. Even though visual comparison of the saddle patches is not scientifically a very accurate method, we found it functional, because in many cases asymmetries were very clear.

Populations

In this study, the transient killer whales were divided into two populations. Most of the Californian transients are normally seen only in Californian waters. West-coast transients are normally met higher up in the coastal waters. There are great differences among transient movements, some are very wide ranging and some have fairly modest home ranges (Ford & Ellis 1999). There are few transient individuals that were regularly encountered in California, but they may have also been seen in British Columbia or in Alaska. From these wide ranging individuals, there is only one with pictures of both sides, and that individual was included in the Californian transient population. Genetically, the west-coast transients and Californian transients are not very distinct, hence they could well be considered members of one population (Ford & Ellis 1999).

Results

In total, 512 killer whales from the six populations from which pictures of left- and right-side saddle patches were available, were visually analyzed. In the six studied populations, there were very few asymmetric individuals in both transient populations and in the offshore population (Table 1). Some Kamchatkan killer whales showed asymmetry between sides. The six studied populations differed in the distribution of the asymmetry classes ($\chi^2 = 103.7$, df = 10, $p < 0.001$). According to the adjusted residuals (Table 1), there were fewer symmetric individuals in the southern resident population than expected. Contrary to the resident populations, two transient populations had more symmetric individuals than expected (Fig. 4 and Table 1).

The Californian transients showed smaller variance ($\sigma^2 = 0.0024$) in the degree of asymmetry, than the southern residents ($\sigma^2 = 0.0177$; comparison of variances $F_{54.85} = 7.33, p < 0.001$). The coefficient of variation (6%) for the Californian transients was only a third of that for the southern residents (18%). The Californian transients had a higher mean level of symmetry (0.819) than the southern residents (0.723; separate variances $t$-test: $t = 6.17$, df = 121.8, $p < 0.001$).

There were 89 measured individuals from the southern residents and 55 from the Californian
transients. Some of these measured individuals were already dead, so these numbers are not the same as the present population size. In the southern resident population, asymmetry was as common in males as in females. From those individuals whose sex was known, roughly half were symmetrical and the other half had small or large asymmetries.

**Discussion**

The killer whale saddle patch pattern is quite symmetric in some populations, but it can also be very asymmetric. Individuals from both resident populations, especially the southern residents, were more asymmetric than those from other populations. They also seem to have more variation in the saddle patch pattern than individuals from the other populations, where the pattern was much less variable. In earlier studies of killer whale color pattern variation, it was noted that the southern resident population differs to some extent from other Pacific killer whales (Baird & Stacey 1988), and also from the north Atlantic and New Zealand populations (Mäkeläinen 1999). The saddle patch patterns in the transient populations seem to vary

**Table 1.** Saddle patch asymmetry of both sides of 512 killer whales from six populations. Each whale’s saddle patches were categorized as symmetric, little asymmetric and asymmetric. Residuals were adjusted so that values smaller than −1.96 or larger than 1.96 indicate significant deviation from the expected value. Asymmetry was more common in both resident populations than in the other analyzed populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of individuals</th>
<th>Symmetric</th>
<th>Little asymmetry</th>
<th>Asymmetric</th>
<th>Estimated population size</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern residents</td>
<td>124</td>
<td>52%</td>
<td>17%</td>
<td>31%</td>
<td>80–90</td>
<td>Ford et al. 2000</td>
</tr>
<tr>
<td>adjusted residual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern residents</td>
<td>129</td>
<td>84%</td>
<td>8%</td>
<td>9%</td>
<td>210–220</td>
<td>Ford et al. 2000</td>
</tr>
<tr>
<td>adjusted residual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>West-coast transients</td>
<td>87</td>
<td>95%</td>
<td>3%</td>
<td>1%</td>
<td>220</td>
<td>Ford et al. 2000</td>
</tr>
<tr>
<td>adjusted residual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Californian transients</td>
<td>78</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>105</td>
<td>Black et al. 1997</td>
</tr>
<tr>
<td>adjusted residual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offshore</td>
<td>33</td>
<td>91%</td>
<td>6%</td>
<td>3%</td>
<td>&gt; 200</td>
<td>Ford et al. 2000</td>
</tr>
<tr>
<td>adjusted residual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kamchatka</td>
<td>61</td>
<td>85%</td>
<td>5%</td>
<td>10%</td>
<td>121</td>
<td>Burdin et al. 2004</td>
</tr>
<tr>
<td>adjusted residual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total count</td>
<td>512</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
little, just as in the north Atlantic killer whales (Mäkeläinen 1999). The measurements results also confirmed the results from the visual analysis that there were more asymmetric individuals in the southern residents than in the Californian transient population. The lack of asymmetry in some populations is also an interesting result. Here, we will discuss genetic and environmental factors as possible explanations for our findings.

Genetic factors

There is a strong relationship between the genomic diversity and developmental stability in Harbor porpoises. The more diverse the genome of the porpoises was, the less skull asymmetry they showed (De Luna et al. 2004). Genetic factors could also affect fluctuating asymmetry in the killer whale color pattern. Even though the transient type killer whales have the highest load of persistent chemicals, they are still able to create a much more symmetrical color pattern than the resident type whales. Genetic diversity was found to be significantly greater in the transients than in the residents (Barrett-Lennard 2000), which may give the transients an advantage in being better able to resist environmental factors causing asymmetry. Transient type individuals with their more fluid social system (Ford & Ellis 1999) may mate more freely among other transients from a wider area. Thus, the probability of inbreeding is smaller and mates are perhaps genetically more diverse which would explain the lower amount of fluctuating asymmetry in transient populations than in resident ones.

DNA analyses have confirmed that residents and transients are genetically distinct and do not interbreed (Ford & Ellis 1999, Ford et al. 2000). They have been suggested to be reproductively isolated from other groups of killer whales as well (Barrett-Lennard 2000, Ross et al. 2000). The size of the southern resident population was around 80–90 individuals. They suffered from capture operations during 1962–1973 (Krahn et al. 2002), which must have had a fragmenting effect on the stable family groups. Mortality was quite high and the population has decreased by 20% between 1995 and 2002 (Grant & Ross 2002). Especially mortality of adult males was high during previous years, which reduced their numbers. If the southern residents are not interbreeding with the other populations, the probability for breeding with relatives is higher in this kind of small population. These factors could explain the high amount of fluctuating asymmetry in the southern resident population as compared with that in the other populations.

Offshore killer whales have been found to be more closely related to residents than to transients. However, they do not seem to mix with residents and transients (Dahlheim et al. 2008, Ford et al. 2000). The low amount of fluctuating asymmetry in the offshore population may be due to the large population size and variability among mates even if they were not interbreeding with other populations.

The killer whale is an abundant species with reduced genetic variation worldwide, especially in mtDNA (Hoelzel et al. 2002). This may point to a bottleneck in the killer whale phylogenetic history (Hoelzel et al. 2002). Heritability of fluctuating asymmetry is normally low, but epistasis may generate additive genetic variation for fluctuating asymmetry that may allow it to evolve in populations subjected to bottlenecks or when rapid environmental changes occur and cause various stress (Leamy & Klingenberg 2005).

Even if the total killer whale population in the Pacific Ocean is large enough to carry ample genetic variation, this may not be the case if the different populations are not interbreeding. Formation of different ecotypes may have caused reproduction to occur within small, socially, behaviorally and geographically divided groups.

Environmental factors

This study showed that the most contaminated killer whale populations were not the most asymmetrical ones. Relative to other marine mammals in the world, total PCB concentrations were found to be very high in the northern and southern residents and in the transient killer whales. The southern residents and transients of British Columbia are considered among the most contaminated cetaceans in the world (Ross et al. 2000). Offshore killer whales are also
shown to contain high levels of PCB and DDT (Dahlheim et al. 2008). Even though the direct effects of high levels of contaminants on free-ranging killer whales are not known, it is known that even small amounts of them cause neurotoxicity, immunotoxicity, reproductive impairment, and endocrine disruption in other species (Reijinders 1986, Ross et al. 2000, Grant & Ross 2002). Environmental factors might have a greater impact on long term survival and immune defense than on appearance and color pattern symmetry (Krahn et al. 2002). Even though we suggest here that genetic factors have a larger effect on killer whale color symmetry, an interaction between genetic and environmental factors could increase fluctuating asymmetry or affect killer whale development and fitness.

There are other environmental stress factors like human disturbance, activities in coastal waters and loud noises, which must have great influence on killer whales. Unfortunately, it is hard to prove or measure those effects on live animals. Resident killer whales may also suffer from declining food resources. At the moment the nutritional status of transients is probably better than of the residents. Oceans have become very noisy places to live in, and loud noises and powerful sonar transmissions are known to cause severe injuries to marine mammals, as well as their mass strandings and deaths (Evans & Miller 2004). During the peak whale watching season, the southern residents are surrounded by boats eight to ten hours every day, and followed by an average of 21 motorboats, peak numbers being 60–70 boats (Erbe 2002). It has been shown that stress caused by loud noise can increase fluctuating asymmetry in the mandibular first teeth in rats (Siegel & Smoolker 1973) and disrupt the calcium transportation which is important for dental development (Siegel & Mooney 1987). Also, neonatal rats that were exposed to audiogenic stress, developed greater asymmetries in some bones (Siegel & Mooney 1978, Mooney et al. 1985). Although the physiological effects of noise in neonatal marine mammals have not been studied, the amount of stress it causes must be large by making communication between individuals more difficult as well as decreasing chances to find prey and rest.

The whales of both resident populations are more asymmetrical than those of the other Pacific Ocean populations and that, as a fitness indicator, gives cause for concern. In the year 2005, the southern population was listed under the Endangered Species Act and possible threats and extinction risks have been analyzed as well (Krahn et al. 2002, Gaydos et al. 2004).

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