Banding studies on six killer whales: an account of C-band polymorphism and G-band patterns

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Abstract. The karyotypes of six killer whales were studied by banding techniques. A striking accumulation of C-heterochromatin has occurred in the Orcinus karyotype. The four telocentric pairs characteristic of the 2n = 44 cetacean karyotypes have become masked due to the accumulation of heterochromatin in the short arms. Conspicuous C-band polymorphism occurred in the materials studied, rendering each specimen karyotypically unique. Owing to the amount and variation of C-heterochromatin in the Orcinus karyotype, access to C-banded karyotypes was essential for the evaluation of the G-band pattern. The G-band pattern of Orcinus was found to be closely similar to that of Stenella. The results indicate an evolution of the Orcinus karyotype from the karyotypes characterizing other delphinids.

In the order Cetacea only two chromosome numbers, 2n = 42 and 2n = 44, have been described (e.g., ARNASON, 1974; DUFFIELD, 1977). The 2n = 42 numbers are found in the odontocete families Physeteridae (ATWOOD and RAZAVI, 1966, ARNASON, 1970; ARNASON and BENIRSCHKE, 1973; DUFFIELD, 1977) and Ziphiidae (ARNASON et al., 1977; DUFFIELD, 1977; BENIRSCHKE and KUMAMOTO, 1978). The mysticete family Balaenidae also has 2n=42 (JARRELL, 1979). The great majority of the cetaceans, both odontocetes and mysticetes, have strikingly similar karyotypes, with 2n = 44. These karyotypes are characterized by four telocentric (t) pairs, two to three subtelocentric (st) pairs, and one or two large plus several smaller submetacentric (sm) pairs. The homologs of the smallest sm pair are frequently attached by their short arms. The metacentric (m) chromosomes are all small, the largest being 4.5–5/n of (A+X). The agreement between the odontocete and mysticete karyotypes was pointed out by ARNASON (1969), who, on the basis of
karyological similarity, advocated the theory of monophyletic origin of the Cetacea.

Among the $2n = 44$ karyotypes only one species, the killer whale, *Orcinus orca*, differs markedly from the general form. Conventionally stained karyotypes of this species have been described earlier (Carr et al., 1966; Horrall et al., 1968; Kulu et al., 1971; Kulu, 1972; Duffield, 1977). Duffield (1977) also presented a G-banded karyotype; the relationship between the killer whale karyotype and the general cetacean karyotype was, however, not resolved.

In the present communication, the karyological relationship between *Orcinus* and other delphinids (*Stenella* and *Tursiops gilli*) is accounted for. Besides conventionally stained karyotypes of each sex, C- and G-banded karyotypes of one specimen are presented. C-banded karyotypes of five additional animals are also given. In order to facilitate the discussion on the relationship between the *Orcinus* karyotype and that of *Stenella* and *T. gilli*, a previously presented karyotype of *S. clymene* (Arnason, 1980) is also included.

Materials and methods

Skin biopsies were collected in Iceland from five living animals, three females and two males. In addition, skin, lung, and cornea samples were taken from one expired male. Chromosome preparations were made in passages 2 to 6. C-bands were made according to the technique of Sumner (1972). For obtaining G-bands, the trypsin-Giemsa method of Wang and Fedoroff (1972) was applied.

In previous studies of cetacean karyology (e.g., Arnason, 1974), the chromosomes of the individual karyotypes were arranged into four groups according to their arm ratios ($r$), as suggested by Levan et al. (1964). The grouping was as follows:

- **m** (metacentric) $r = 1.00-1.67$
- **sm** (submetacentric) $r = 1.67-3.00$
- **st** (subtelocentric) $r = 3.00-7.00$
- **t** (telocentric) $r = 7.00-\infty$

The *Stenella* karyotype presented is arranged according to this system. The present chromosomal arrangement of the *Orcinus* karyotype is in accordance with the results of the banding analyses and enables a direct comparison with the *Stenella* karyotype. An arrangement of the killer whale karyotype based strictly on arm ratios was therefore not applied.

Results

Unbanded karyotypes of both sexes are shown in fig. 1. The main characteristics of the $2n = 44$ cetacean karyotypes, the four t pairs, are not present in the killer whale karyotype. After the construction of a $t^e$ group, comprising four pairs with quite variable arm ratios, the characteristics of the general cetacean karyotype did, however, emerge. The arrangement in the m, sm, and st groups is in agreement with that of *Stenella* and *Tursiops* (Arnason, 1974, 1980). The X is between 4.5 and 5% of (A+X); the Y is minute. Two pairs frequently involved in chromosome attachment are found in the killer whale karyotype. These pairs are one of the smallest of the complement, sm9, and one $t^e$ pair. In other $2n = 44$ cetacean karyotypes, only the homologs of the smallest pair are frequently associated. In the killer whale karyotype, the homologs of both pairs associated in all possible combinations. Associations involving other chromosomes were not observed. In the killer whale karyotype, the satellite fibers of sm9 extend from the long arm (q); in other $2n = 44$ karyotypes the fibers extend from the short arm (p). In the $t^e$ group, the pair involved in associations was designated $t^e 4,
while the order of the other three pairs were based on the length of the long arm. In t<sup>8</sup> 1, the long arm is considerably larger than in the other t<sup>8</sup> pairs.

In the unbanded male karyotype (fig. 1a) size heteromorphism can be seen in a few pairs, notably in sm4 and t<sup>8</sup>l. Heteromorphisms in the female karyotype (fig. 1b) are less conspicuous. The pairing of both karyotypes was carried out after the C-banded karyotypes had become available. Without access to banded karyotypes, the heteromorphisms had presumably become masked in most instances.

Figure 2 shows C-banded karyotypes representing the same animals as in fig. 1. The amount of C-heterochromatin in the killer whale is much greater than in other delphinids previously studied (Arnason, 1974, 1980). As in other cetacean karyotypes, the C-heterochromatin occurs predominantly in interstitial and terminal chromosome positions. The interstitial bands are present in the larger chromosomes, and an
interstitial band also characterizes t1. Centromeric heterochromatin is sparse. In the t^e chromosomes the entire p arms are C-band positive. The satellites and satellite fibers in t^4p and sm9q which are involved in chromosome associations are C-band negative, but extend in both instances from C-band-positive segments.

In the male δ1 karyotype (fig. 2a), conspicuous C-band heteromorphosis occurs in sm2q, sm3p, sm4p, sm9q, and t^1p. In the female δ1 karyotype (fig. 2b), the most prominent C-band heteromorphisms are found in sm2q, sm3p, and q.

C-banded karyotypes of two more males are shown in fig. 3. In δ2, C-band heteromorphisms are not particularly marked, but, nevertheless, evident in sm2q, sm6p, t^3p, and t^4p. In δ3, striking heteromorphisms occur in st1 and t^1p. In this specimen, the short arms of t^4 are small and only faintly C-band positive. Bar: 10 μm.

In the δ2 karyotype (fig. 4), the heteromorphisms in t^3p and t^1p are extraordinary. Prominent heteromorphosis is also seen in sm1q, sm3q, and sm4p.

The C-banded karyotype of δ3 (fig. 5c) shows striking C-band heteromorphism in the t^e chromosomes. The long arms of st1 are also heteromorphic, even though in this particular preparation the differences between the arms are exaggerated in comparison with other cells from the same animal. The heteromorphisms in sm2, sm5, sm9, and st2 are also notable. Figure 5b shows a G-banded karyotype of the same specimen. The G-band pattern permitted unequivocal pairing of the whole complement, even
Fig. 5. Banded karyotypes of ♀3: (a) C-bands; (b) G-bands. C-band heteromorphism occurs in most pairs. The heteromorphism is particularly prominent in the t⁶ and st chromosomes and in sm9q. The G-band pattern permits an unequivocal identification of almost the whole complement. The G-bands corresponding to the C-bands are faintly stained; the differentiation is limited or nonexistent. Bar: 10 μm.

though the patterns of m2 and m3, and m4 and m5, respectively, are similar. The heteromorphisms of the C-banded karyotype are reflected in the G-band pattern; this is particularly striking in the short arms of the t⁶ group and in sm9q. The C-band positive regions are lightly stained in the G-banded preparations, and the differentiation

Fig. 6. A G-banded karyotype of *Stenella clymene*, male, 2n = 44. G-band homologies between the *Stenella* and *Orcinus* karyotypes are evident in a great majority of the pairs. Bar: 10 μm.

in these segments is very limited or even nonexistent.

As mentioned above, a G-banded karyotype of *S. clymene* (fig. 6) has been presented previously (Arnason, 1980). Apart from differences caused by the different accumulation of heterochromatin, the similarities of G-band patterns of *Orcinus* and *S. clymene* are evident.

**Discussion**

The relationship between the killer whale karyotype and the other cetacean 2n = 44 karyotypes has been enigmatic. Duffield (1977), comparing a G-banded *Orcinus* karyotype with the 2n = 44 karyotypes, registered apparent chromosomal rearrangements within a number of the metacentric and submetacentric killer whale chromosomes. The karyotype was reported as having 11 unique pairs for which homology with other cetaceans could not be clearly specified. The karyotype was considered
equally distinct from both the mycticete and odontocete species clusters, and the interpretation was suggested that *Orcinus* represented an early radiation.

In the present study, the facility with which C-bands were generally produced was of great help for the comparison with the other delphinid karyotypes, but even without access to C-banded karyotypes, the similarity between the G-banded *Orcinus* karyotype, on the one hand, and the *Stenella* and *T. gilli* karyotypes, on the other, was found to be quite obvious. According to Árnason (1974), the *Stenella* and *T. gilli* karyotypes have identical G-band patterns. This conclusion, reached after comparing original materials, was clearly stated in the paper referred to. Duffield (1977), comparing the reproduced material, scored only 17 identical pairs between *Stenella* and *T. gilli*. Additional material representing *S. clymene* (Árnason, 1980) has confirmed the earlier stated identity of the *Stenella* and *T. gilli* karyotypes.

A comparison of the G-band pattern of *Orcinus* (fig. 5b) with that of *S. clymene* (fig. 6) shows that, apart from differences caused by different accumulation of heterochromatin, there exist great similarities between the two karyotypes. In the m group, five out of six pairs showed identical banding. Identities were scored in five out of nine sm chromosomes. The two st pairs correspond, as do the q arms of at least three of the t and t* pairs. The X’s of both species correspond to the X generally found in cetaceans. In addition, there is reason to assume that the smallest pair, sm9, in *Stenella* corresponds to sm9 in the *Orcinus* karyotype, the differences presumably being caused by different accumulation of heterochromatin in the region adjacent to the nucleolus organizing region (NOR) site; in both species sm9 shows a Ag-positive reaction (Árnason, in preparation). In the killer whale karyotype, five pairs, m4, sm3, sm5, sm8, and t*4, were not scored as sharing great similarities with the m4, sm3, sm5, sm8, and t4 pairs in the *Stenella* karyotype. It may, nevertheless, be argued that some similarities exist between all pairs with corresponding designations, except sm3.

The killer whale karyotype can be summarized as follows: Conventionally stained *Orcinus* karyotypes differ markedly from the general 2n = 44 cetacean karyotypes. C-band studies showed that these differences are mainly due to the accumulation of C-heterochromatin in the killer whale karyotype; the main characteristics of the general karyotype, the four t pairs, have thus become masked. A comparison between G-banded karyotypes showed that great similarities exist between the karyotypes of *Stenella* and *T. gilli* and that of *Orcinus*.

As mentioned above, Duffield (1977) suggested that the *Orcinus* karyotype represented an early radiation. The great accumulation of C-heterochromatin in the *Orcinus* karyotype as compared with other delphinid karyotypes, does not support this view. The accumulation of C-heterochromatin is considered to be a secondary characteristic and, thus, represents a recently evolved trait. Also, the presence of two pairs with NOR sites in *Orcinus*, as compared with only one in the general cetacean karyotype (Árnason, in preparation), suggests that the *Orcinus* karyotype has rather evolved from the 2n = 44 karyotype.

The first cetacean chromosome studies based on long-term cell culture were carried out by Walen and Madin (1965), who described the karyotypes of two delphinids,
the bottle-nosed dolphin and the short-fin pilot whale. As early as this study, size heteromorphism was reported. Later, the frequently occurring heteromorphisms in the cetacean karyotypes were found to be attributable to striking C-band polymorphisms, which actually rendered each animal karyotypically unique (Árnason, 1974). Additional materials (Jalal et al., 1974; Árnason et al., 1977, 1978; Duffield, 1977) have further underlined the general occurrence of these features. The amount of heterochromatin and the polymorphisms observed were especially marked in the mysticete genus Balaenoptera. Árnason et al. (1978), reporting conservatism and chromosomal localization of satellite DNA in the baleenopterids, discussed the effect C-band heteromorphism might have on meiotic pairing. It was assumed that within the extremes in C-band sizes, there existed a more or less continuous variation, with different size classes dominating in different populations. It is conceivable that in such a situation, euchromatin-heterochromatin overlappings of various degrees between homologous chromosomes will be more or less ubiquitous at the time of meiotic pairing. In the regions of euchromatin-heterochromatin overlapping crossing-over would be excluded due to lack of parity, but parity would be achieved at a distance from the euchromatin-heterochromatin overlapping, probably due to looping out of the unpaired portion. The implication would be that, in general, crossing-over in the vicinity of heterochromatin would be reduced and the coupling in each case would be related to the heteromorphism involved. Since homologous chromosome ends are fixed with respect to each other in the meiotic bouquet formation, the effects of C-band heteromorphisms on pairing would be analogous, irrespective of whether the C-heterochromatin is terminal, interstitial, or centromeric. The genetic effects of the system outlined would be that genes located close to heterochromatin would be collectively inherited and selected, whereas more distantly located genes would be assorted independently.

In the present material, the amount of heterochromatin is much greater than in other delphinids studied; in fact, the amount is fully comparable to that of the genus Balaenoptera. The amplitude of C-band polymorphism in the killer whale is similar or even greater than that encountered in the baleenopterids. For obvious reasons, genetic studies of recombination cannot be carried out in cetacean materials, and cytological studies of meiosis are unfortunately hampered by the considerable difficulties associated with the collection of samples.

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