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Whistle Matching in Wild Bottlenose Dolphins (*Tursiops truncatus*)

Vincent M. Janik

Dolphin communication is suspected to be complex, on the basis of their call repertoires, cognitive abilities, and ability to modify signals through vocal learning. Because of the difficulties involved in observing and recording individual cetaceans, very little is known about how they use their calls. This report shows that wild, unrestrained bottlenose dolphins use their learned whistles in matching interactions, in which an individual responds to a whistle of a conspecific by emitting the same whistle type. Vocal matching occurred over distances of up to 580 meters and is indicative of animals addressing each other individually.

Bottlenose dolphins show many cognitive and communicative skills that are rare among animals. They are capable not only of generalizing rules, developing abstract concepts and syntactic understanding in an artificial communication system (*1*), but also of vocal learning, i.e., the ability to modify the structure of a vocal signal as a result of experience with those of other individuals (*2*). Although extensive studies in nonhuman primates have not been able to present convincing evidence for vocal learning, this prerequisite for the evolution of spoken language has been demonstrated with much less research effort in bottlenose dolphins (*2*). Dolphins are capable of imitating new sounds accurately at their first attempt, and they keep this ability throughout their life (*3*). Vocal learning is also an important factor in the ontogeny of an individually distinctive signature whistle that each individual develops in the first few months of its life (*4*). Studies on captive individuals have shown that signature whistles are primarily used if animals are out of sight of each other, and they are therefore thought to function in group cohesion and individual recognition (*5*–*7*). However, because bottlenose dolphins are capable of vocal learning, individual signature whistles can be found in the repertoire of more than one individual in captive dolphins (*6*, *8*).

I investigated whether such shared whistles occur in matching whistle interactions between wild dolphins, a phenomenon indicative of their use in addressing specific individuals. Matching interactions were defined as an occurrence in which two whistles of the same type produced by separate individuals occurred within 3 s of each other.

There is often a clear effect of observer presence on dolphin behavior when methods such as tagging or boat pursuits are applied (*6*, *9*). I used a noninvasive passive acoustic localization technique (*10*) to locate calling bottlenose dolphins (*11*). This method uses the differences in the time of arrival of the same sound at different widely spaced hydrophones. Signals from different recording channels were cross-correlated to determine the difference in the time of arrival of a sound at the two corresponding hydrophones. The time-of-arrival comparisons of three pairs of hydrophones then result in three hyperbolas of possible sound source locations. These hyperbolas intersect at the true location of the whistling dolphin. This analysis was conducted with SIGNAL software (Engineering Design, Belmont, Massachusetts). Recordings were conducted in the Kessock Channel of the Moray Firth, Scotland. All data were acquired from the shore, so that no boats or humans were present around the animals.

Vocal interactions between individuals were identified by comparing the distance of the source locations of two successive whistles (minus twice the maximum localization error of 13 m) with the distance that a bottlenose dolphin could travel at its maximum reported swimming speed of 7.5 m/s (*12*) in the interwhistle interval. If the distance between two whistle sources could not have been covered by one individual in the time interval between those whistles, they must have been produced by different individuals.

Five naïve human observers were used to rate the similarity of each whistle interaction using only the extracted contours (*13*) of the whistles; this method is more reliable than computer-based methods that have been used in dolphin whistle studies (*14*). They were allowed to rate whistle similarity on a scale from 1 (=dissimilar) to 5 (=similar). The scores of the different observers were significantly similar (Kappa = 0.34, $z = 16.9$, $P < 0.00001$). Only whistle pairs that reached an average score of more than 3.0 were considered to be matching interactions (*15*).

In a total recording time of 258 min and 43 s from seven different days in July and August 1994 and 1995, a total of 1719 whistles was recorded. These recordings were made with an average of 10 animals present in the channel (quartiles: 7, 10, and 15). Independent counts conducted by a second observer from a higher observation point using binoculars showed that these counts were highly accurate. I could not identify individuals in this study, but a photo-identification study showed that at least 14 different individuals were using this area on a regular basis and that occasionally groups of more than 20 animals were present (*16*). Nine hundred ninety-one of the recorded whistles had a sufficient signal-to-noise ratio on all hydrophones for their source location to be determined. In this sample, 176 whistle interactions were found, of which 39 were classified as matching interactions (Fig. 1). In both matching and nonmatching interactions, 80% of the interwhistle interval was less than 1 s. The mean distance between matching individuals was 179 m (standard error: 22.8 m); the maximum was 579 m. Distances between animals in matching interactions were significantly smaller than those of animals in nonmatching interactions (Kolmogorov-Smirnov Two-Sample Test, two-tailed, $D = 0.291$, $P < 0.025$) (Fig. 2). A randomization test (*17*) showed that this number of matching interactions was signifi-

School of Biology, University of St. Andrews, Bute Building, Fife KY16 9TS, UK, and Lighthouse Field Station, Aberdeen University, Cromarty, Ross-shire IV11 8YJ, UK. Present address: Woods Hole Oceanographic Institution, Biology Department, Woods Hole, MA 02543, USA.

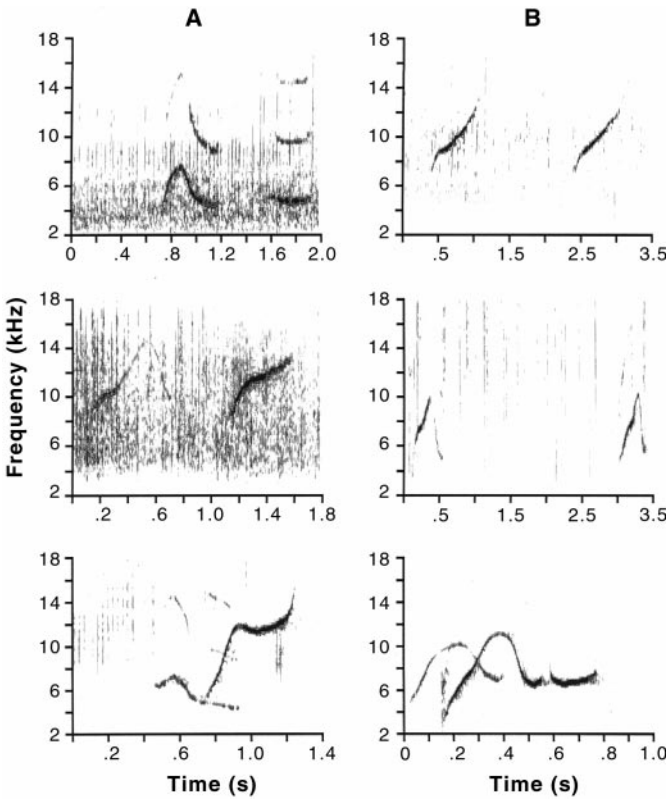

Fig. 1. Spectrograms of three examples of nonmatching (**A**) and matching (**B**) whistle interactions. The whistling dolphins were 55, 74, and 29 m apart (from top to bottom) in (A) and 158, 204, and 379 m apart in (B). Average similarity scores were 2, 2.4, and 1.4 in (A) and 4.2, 4.2, and 3.4 in (B). Human judges inspected frequency contours (i.e., line representations of the frequency modulation of the fundamental frequency; time resolution: 5 ms; frequency resolution: 200 Hz) rather than spectrograms on a more detailed scale than shown here. The actual size of each contour graph was 10 cm by 12 cm (*25*).

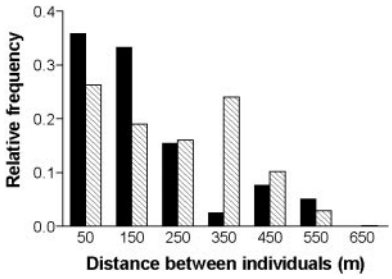

Fig. 2. Distributions of the distances between dolphins in matching (solid bars) and nonmatching (hatched bars) whistle interactions.

cantly greater than expected if all animals were calling independently of each other (999 runs; observed proportion: 0.04; chance proportion: 0.018; $P = 0.001$) (*15*). The assumptions of this test were that each of the 10 animals present had at least one individually distinctive signature whistle type and that it could copy each of the signature whistle types used by the other nine individuals. Thus, matching was judged to occur in a repertoire of 10 shared but learned whistle types. These assumptions are based on the findings from previous studies that each individual bottlenose dolphin develops its own distinctive signature whistle type (*5*) and that a bottlenose dolphin can copy new whistle types at the first attempt (*3*). Furthermore, it was assumed that each dolphin whistled at the same rate. These assumptions are conservative, as an increase in whistle types or unequal whistling rates would make matching less likely to occur by chance. Furthermore, most matching interactions occurred when there were more than 10 animals in the channel, which also makes matching less likely to occur by chance.

The number of all whistle interactions including nonmatching interactions was not significantly different from chance (999 runs; observed proportion: 0.18; chance proportion: 0.18; NS). Most matching interactions only involved two animals, each producing just one whistle. However, in three cases, the first animal produced another matching whistle after it had been matched, and in one case, two matching interactions followed each other within 5 s. In three cases, matching interactions involved three individuals (Fig. 3). Matching whistle interactions were found on all seven days for which recordings were analyzed.

With the methods used here, animals swimming within 26 m of each other could not be identified as different individuals. Thus, it is possible that matching interactions are more common than shown here. Indeed, 50 overlapping whistles from one location could be found in the sample. However, because individual cetaceans have been reported to produce two whistles simultaneously (*18*), I excluded these cases from the analysis. Whistles in seven matching interactions of clearly separate individuals, however, also overlapped, a behavior that has been linked with aggression in birds (*19*–*21*).

These results show that bottlenose dolphins use their learned whistles in matching interactions, most likely to address each other. The character of such addressing might be either aggressive or affiliative. However, matching could also signal alliance membership to third

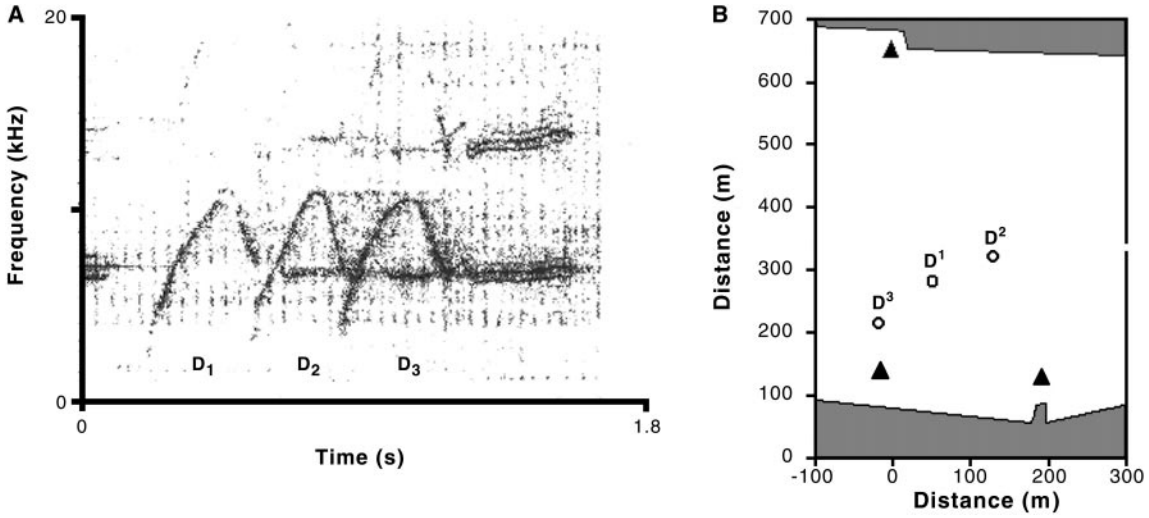

Fig. 3. A matching whistle interaction that involved three individuals. (**A**) Spectrogram of the produced whistles. (**B**) Plot of the array geometry with the locations of each of the dolphins that produced whistles D_1, D_2, and D_3 in (A). Gray areas at the top and the bottom of the plot represent the shoreline. Circles, animals; triangles, hydrophones (*25*).

parties or be used to prevent deception of third parties by a whistle-copying dolphin. Although vocal matching is common in birds (*22*), bottlenose dolphins are the only nonhuman mammals in which matching interactions with learned signal types have been found. The occurrence of such matching or labeling has been hypothesized to have been an important step in the evolution of human language (*23*, *24*). The results presented here show that reaching that step can be achieved in very different environments.

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PAX8-PPARγ1 Fusion in Oncogene Human Thyroid Carcinoma

Todd G. Kroll,[1]* Pasha Sarraf,[2] Lorenza Pecciarini,[1] Chang-Jie Chen,[1] Elisabetta Mueller,[2] Bruce M. Spiegelman,[2] Jonathan A. Fletcher[1,2,3]*

Chromosomal translocations that encode fusion oncoproteins have been observed consistently in leukemias/lymphomas and sarcomas but not in carcinomas, the most common human cancers. Here, we report that t(2;3)(q13;p25), a translocation identified in a subset of human thyroid follicular carcinomas, results in fusion of the DNA binding domains of the thyroid transcription factor PAX8 to domains A to F of the peroxisome proliferator–activated receptor (PPAR) γ1. PAX8-PPARγ1 mRNA and protein were detected in 5 of 8 thyroid follicular carcinomas but not in 20 follicular adenomas, 10 papillary carcinomas, or 10 multinodular hyperplasias. PAX8-PPARγ1 inhibited thiazolidinedione-induced transactivation by PPARγ1 in a dominant negative manner. The experiments demonstrate an oncogenic role for PPARγ and suggest that PAX8-PPARγ1 may be useful in the diagnosis and treatment of thyroid carcinoma.

Chromosomal translocations encoding fusion oncoproteins are common in leukemias/lymphomas and sarcomas (*1*) but have been identified in only a single adult human (thyroid papillary) carcinoma. Compared with fusion oncoproteins in noncarcinomas, those in thyroid papillary carcinoma occur at relatively low frequency and are derived from several distinct gene fusion events, the most common of which result from subtle chromosomal inversions (*2*). Most cytogenetic abnormalities characterized in carcinomas to date are deletions that remove growth-restraining tumor suppressor genes. These findings imply (i) that most human carcinomas develop through translocation-independent events, or (ii) that most carcinoma translocations are subcytogenetic alterations that are difficult to detect in complex carcinoma karyotypes (*3*). Distinction between these alternatives is important because carcinomas constitute up to 90% of human cancers.

We have determined the genetic consequences of t(2;3)(q13;p25), a chromosomal translocation identified in human thyroid follicular carcinomas. Three consecutive thyroid follicular carcinomas (*4*) karyotyped in our laboratory exhibited t(2;3)(q13;p25), which has been reported previously in thyroid follicular tumors, including one with lung metastases (*5*). We first mapped the 3p25 and 2q13 translocation breakpoints using interphase fluorescence in situ hybridization (FISH) (*6*). The 3p25 breakpoint region was narrowed to ~600 kb and was bordered by yeast artificial chromosomes (YACs) 753f7 (telomeric) and 903e6 (centromeric) (Fig. 1A). Hybridization with flanking YACs 753f7 and 932f3 confirmed 3p25 rearrangements in tumor but not normal cells (Fig. 1B). The 2q13 breakpoint was localized within overlapping YACs 989f12 and 896a8 (Fig. 2A) to a region containing *PAX8*, which encodes a paired domain transcription factor essential for thyroid development (*7*). A *PAX8*-containing bacterial artificial chromosome (BAC), 110L24, crossed the 2q13 breakpoint and cohybridized with 3p25 YAC 753f7 (Fig. 2B), consistent with involvement of *PAX8* and a 3p25 partner in the translocation.

To identify the 3p25 partner, we performed rapid amplification of cDNA ends (RACE) using 5′ *PAX8* primers (*8*). Sequence analysis of RACE products from t(2;3)-positive follicular carcinomas (*8*) revealed in-frame fusion of *PAX8* to the peroxisome proliferator–activated receptor γ (*PPAR*γ) gene (Fig. 3A). *PPAR*γ has been mapped to 3p25 (*9*), and a PPARγ-containing BAC, 321f13, crossed the 3p25 breakpoint and cohybridized with 2q13 YAC 989f12

[1]Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA, and Harvard Medical School, Boston, MA 02115, USA. [2]Dana-Farber Cancer Institute and Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA. [3]Departments of Pathology and Pediatric Oncology, Children's Hospital, Boston, MA 02115, USA.

*To whom correspondence should be addressed. E-mail: tkroll@rics.bwh.harvard.edu; jfletcher@rics.bwh.harvard.edu