Hematology distinguishes coastal and offshore forms of dolphins (Tursiops)

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Hematology distinguishes coastal and offshore forms of dolphins (*Tursiops*)

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Bottlenose dolphins, *Tursiops truncatus*, can be separated into coastal and offshore ecotypes based upon hemoglobin levels, packed cell volumes, and red blood cell counts, the offshore form having higher values for all three measures. Captive-bred crosses between coastal and offshore types produce animals with intermediate hematologic profiles suggesting a significant genetic basis for these differences.

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Les dauphins à gros nez *Tursiops truncatus* se distinguent en deux écotypes, dans la zone côtière et au large, selon leur concentration en hémoglobine, le volume de leur hématocrite et le nombre de leurs globules rouges; ces valeurs sont toujours plus grandes chez les dauphins du large. Des croisements en captivité de dauphins des deux écotypes produisent des animaux à profils hématologiques intermédiaires, ce qui indique que des facteurs génétiques sont en grande partie responsables des différences entre les deux écotypes.

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Introduction

Tursiops truncatus, the bottlenose dolphin, is the best known of the small whales largely owing to their popularity as trained oceanarium exhibits throughout the world (Ridgway and Benirschke 1977: Cornell and Asper 1978) and to their extensive use in research on sonar and bioacoustics (Busnel and Fish 1980). Different morphological types of *Tursiops* have been described (Tomilin 1957; Hershkovitz 1966; Nishiwaki 1972; Rice 1977; Ross 1977) and it has been suggested that separate coastal (inshore) and offshore varieties may exist (Mitchell 1975). Recently, three distinguishable forms have been proposed in the eastern North Pacific, one coastal and two offshore (Walker 1981), and it is probable that a differentiation of coastal and offshore types will be characteristic of the Atlantic as well (Leatherwood *et al.* 1976; Ross 1977). Interested in developing ways of evaluating genetic differences between these proposed morphological variants, we have been examining chromosomal heteromorphisms and protein electrophoretic markers in captive *Tursiops* collected from Atlantic and Pacific areas, in capture–release samplings of coastal Atlantic *Tursiops* and in Atlantic and Pacific *Tursiops* in captive breeding programs (Duffield *et al.*, in preparation[1]; Cornell *et al.*, in preparation[2]). As a corollary to these studies, differences in the hematologic profiles of some of these *Tursiops* were noted. Preliminary comparison of reported hematologic values (Medway and Geraci 1964; Ridgway and Johnson 1966; Lenfant 1969; Ridgway *et al.* 1970) also suggested that differences in hemoglobin concentration, packed cell volume, and red blood cell count might well exist among *Tursiops* from different areas. It has been shown that intergeneric differences in blood volume, packed cell volume (PCV), and hemoglobin (Hb) concentration exist between small toothed whale species exhibiting differences in activity level, habitat, and diving capacity (Ridgway and Johnson 1966). The faster swimming, deep-diving, offshore species *Phocoenoides dalli* (blood volume, 143 mL/kg body weight; Hb, 20.3 g/100 mL blood; PCV, 57%) had higher values in all three categories than an intermediate offshore species, *Lagenorhynchus*

[1]Duffield, D. A., D. K. Odell, E. D. Asper, and S. Searles. Biochemical variability in Indian River *Tursiops*; application to the study of population dynamics.

[2]Cornell, L. H., E. D. Asper, J. E. Antrim, and S. Searles. Sea World: *Tursiops* breeding program.

obliquidens (108 mL/kg; 17.0 g/100 mL; 53%), the lowest values being those of coastal Atlantic *Tursiops* (71 mL/kg; 14.4 g/100 mL; 45%). The apparent correlation of these values with oxygen demand and diving habits of the three species suggested to us that hematological screening might identify animals from coastal and offshore populations of *Tursiops*.

Methods

We have compared hematological values of 70 Atlantic *Tursiops* and 35 Pacific *Tursiops* maintained at Sea World facilities in San Diego and Florida. Six captive-bred calves (Atl:Atl and Atl:Pac crosses) born at these parks have also been examined. Detailed long-term records of hematology were available from routine health checks made at regular intervals. Only data from apparently healthy animals, as determined by behavior and absence of abnormal values for clinical measures, such as white blood cell counts, serum enzymes, and blood chemistry determinations, were included in the study. All blood samples were drawn by fluke venipuncture (Ridgway 1965) and analyzed in the Sea World Clinical Laboratory. Hemoglobin (Hb, grams per 100 mL blood), packed cell volume (PCV, percent), and red blood cell number (RBC 10^6 per cubic millimetre) were compared. Red blood cell counts and PCV were done on a Coulter ZB1 (Coulter Electronics Inc., Irvine, CA), calibrated daily with Coulter normal and abnormal controls. Hemoglobin was determined using a Coulter hemoglobinometer also calibrated daily against standards.

The dolphins were stratified by capture site: (*i*) Atlantic, coastal (n = 70); (*ii*) Pacific, offshore (Walker 1981) (n = 6), captured near the San Clemente and Santa Barbara Islands off southern California; (*iii*) Pacific coastal (Walker 1981) (n = 29), captured in the Gulf of Baja California at Puertocitos or San Felipe, or along the ocean side of the Baja coast at San Quentin; and (*iv*) captive-bred dolphins (n = 6), including those resulting from the breeding of animals from coastal and offshore capture sites. Hematologic values for each animal were averaged over the period of time in captivity (<1–17 years; the majority being in captivity from 6 to 10 years).

Results and discussion

There were no apparent sex- or age-related differences in Hb, PCV, or RBC and there was no evidence of long-term acclimational effects for these values in any of the *Tursiops* studied.

Individual animal means for Hb, PCV, and RBC were used in a *K*-means clustering analysis (Anderberg 1973) to determine whether distinct hematologic clusters could be identified. The Atlantic coastal vs. the Pacific offshore animals formed independent homogeneous clusters with no overlap. Mean Hb, PCV, and RBC were significantly higher in the Pacific offshore animals than in the Atlantic coastal animals (Table 1, A). The distinction in hematological profile between the Atlantic coastal and Pacific offshore *Tursiops* was apparent whether initial capture values or long-term captivity averages were compared.

Hematologic values for captive-bred calves of Atlantic × Atlantic crosses (n = 4, sampled from 1 year of age) were the same as those of the wild-caught Atlantic coastal animals (Table 1, B). However, Atlantic × Pacific cross-bred calves (n = 2, Table 1, B) exhibited distinctly intermediate values for Hb, PCV, and RBC, suggesting a significant genetic component to the observed hematological differences between these forms. The difference in Hb, PCV, and RBC profiles between the Atlantic coastal vs. Pacific offshore animals and the intermediate values for the Atl:Pac cross-bred calves are illustrated in Fig. 1.

Electrophoretic differentiation of Atlantic and Pacific *Tursiops* stocks has previously been noted (D. A. Duffield, personal observation). Therefore, hematologic values of *Tursiops* from Pacific coastal capture sites were compared with Atlantic coastal and Pacific offshore values (Fig. 2) to determine whether the observed differences in hematologic profiles represented stock differentiation between Atlantic and Pacific *Tursiops* or whether they might be indicative of coastal vs. offshore ecotypic differentiation within a particular ocean system. Cluster analysis of the hematologic values for the Pacific coastal category (Table 1, C) identified three distinctive groups; a "low" cluster with values equivalent to those of the Atlantic coastal group, a "high" cluster with values equivalent to those of the Pacific offshore, and an "intermediate" cluster with values like those of the cross-bred calves, falling between those of the coastal and offshore types. The "low" cluster demonstrates the presence in the Pacific of *Tursiops* exhibiting a "coastal" hematologic profile similar to that of the Atlantic coastal animals. This suggests that the hematological differences which exist in *Tursiops* define coastal vs. offshore hematologic ecotypes, not just Atlantic–Pacific stock differences. Furthermore, the presence in the Pacific coastal area of *Tursiops* with high, offshore hematologic profiles as well as the low, coastal profiles suggests that this particular region contains a mix of animals of both ecotypes, either as transients or as resident populations. That individuals of intermediate hematologic profile similar in values to the captive-bred crosses between the two ecotypes are also found here would serve to indicate that reproductive exchange between coastal and offshore forms is occurring in this area. The intermediate hematologic cluster in the Pacific "coastal" sample could itself be clustered, although with less distinction, into three groups; one closer to the low category, one intermediate, and one closer to the high. If the Pacific coastal area sampled were one in which a high degree of reproductive exchange between offshore and coastal populations were occurring, one would expect continued back-crossing with coastal and offshore forms to produce these additional clusters.

TABLE 1. Hematologic values

	Hb (g/100 mL), mean ± 95% confidence limits (range)*	PCV (%), mean ± 95% confidence limits (range)*	RBC (× 10^6/mm^3), mean ± 95% confidence limits (range)*
(A) Atlantic coastal			
Tursiops (*n* = 70)	14.5±0.4	42±1	3.61±0.1
	(13–16)	(37–47)	(3.11–4.14)
Pacific offshore			
Tursiops (*n* = 6)	18.4±0.9	52±3	4.49±0.3
	(18–20)	(47–56)	(4.05–4.83)
(B) Calves (bred in captivity)			
Atl × Atl (*n* = 4)	14.4±0.4	40±2	3.56±0.2
	(14–15)	(39–42)	(3.45–3.70)
Atl × Pac (*n* = 2)	16.0	45	3.94
	(16)	(43,47)	(3.86,4.03)
(C) Pacific coastal sites			
Low (*n* = 6)	14.1±0.7	41±3	3.64±0.4
	(13–15)	(38–46)	(3.13–4.18)
Intermediate (*n* = 11)	16.5±0.6	45±1	4.03±0.1
	(16–17)	(43–48)	(3.86–4.29)
High (*n* = 12)	18.5±0.6	48±1	4.52±0.1
	(17–20)	(45–51)	(4.23–4.79)

*Range of individual means.

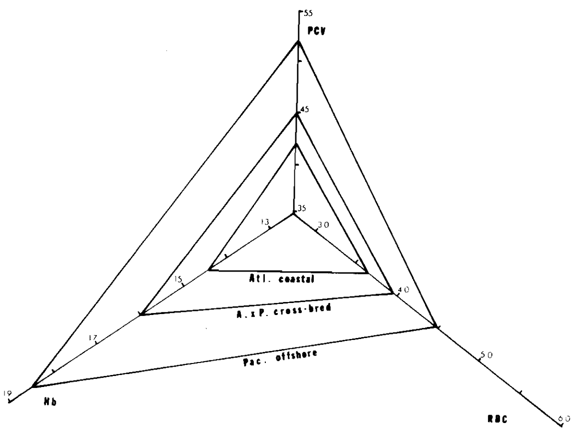

FIG. 1. To illustrate the distinction in hematologic profiles, mean Hb, PCV, and RBC are represented for Atlantic coastal *Tursiops* (Atl. coastal) and Pacific offshore *Tursiops* (Pac. offshore). An intermediate profile is seen in captive-bred crosses (A. × P. cross-bred).

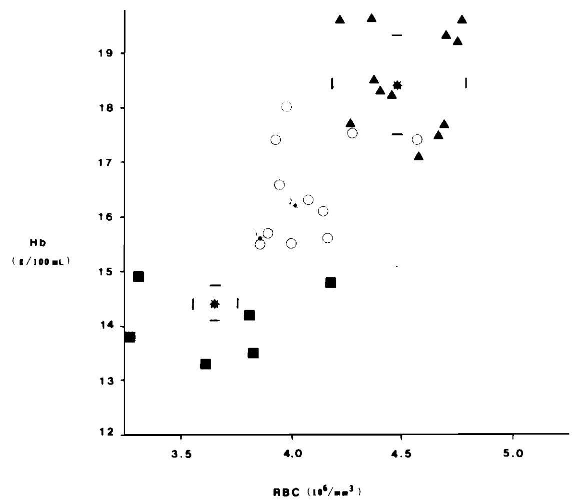

FIG. 2. Hemoglobin vs. RBC for *Tursiops* from Pacific coastal capture sites: solid triangles are *Tursiops* of the "high" cluster (see Table 1, C), solid squares are *Tursiops* of the "low" cluster and open circles are *Tursiops* of the "intermediate" cluster. Solid stars represent the mean value for Atlantic coastal *Tursiops* (lower star; 95% confidence limits in brackets) and Pacific offshore *Tursiops* (upper star; 95% confidence limits in brackets). The two cross-bred calves are indicated by the light stars.

In captivity there was no consistent difference between coastal and offshore forms in the amount of hemoglobin per cell (mean corpuscular hemoglobin = Hb/RBC), the amount of hemoglobin per packed cell volume (mean corpuscular hemoglobin concentration = Hb/PCV), or in the mean cell volume (mean corpuscular volume = PCV/RBC), although there was a "trend" towards lower mean cell volume in the offshore forms. The observed differences in the hematologic profiles of the two forms seem largely due to differences in the numbers of circulating red blood cells. An Atlantic coastal *Tursiops* and a Pacific offshore *Tursiops* trained to deep dive unrestrained (Ridgway 1972) both showed an increase in the numbers of circulating RBC's during the period of diving from values near the hematologic means for these two forms.

The increase was far greater in the animal of offshore origin (coastal high, 4.32×10^6; offshore high, 6.75×10^6) and was associated with a significantly lower mean cell volume (MCV) (coastal, MCV = 131 fL; offshore, MCV = 90 fL). Adaptation in the offshore form was therefore accompanied by formation of large numbers of small circulating red blood cells. This response was not evident in the coastal *Tursiops*, possibly indicating different physiological responses made by these two types to deep diving.

In conclusion, coastal and offshore hematological ecotypes can be distinguished in the bottlenose dolphin (*Tursiops*). Hemoglobin concentration, packed cell volume, and red blood cell counts are significantly higher in the offshore type. Captive-bred crosses between the coastal and offshore ecotype produce calves with intermediate hematologic profiles for all three measures, indicating a significant genetic basis for the ecotypic differences in these measures. These findings suggest that marine mammal species such as *Tursiops* face selective pressures relative to oxygen storage and delivery strong enough to establish detectable genetic differences in hematology between coastal and offshore populations. We present these ideas in the hope that they will stimulate the field application needed to define the precise role which hematological differentiation plays in the development and maintenance of ecotypes in diving mammals.

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