CHROMOSOMAL CHARACTERIZATION OF FOUR ANTARCTIC PROCELLARIIFORMES

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SUMMARY

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The karyotype and C-banding patterns of Southern Giant Petrels *Macronectes giganteus*, Cape Petrels *Daption capense*, Black-bellied Storm Petrels *Fregetta tropica* and Wilson's Storm Petrels *Oceanites oceanicus* were analyzed. To obtain metaphases, the direct culture technique was used on feather bulbs and lymphocytes of peripheral blood of 34 specimens. Both Southern Giant and Cape Petrels share 2n = 80 chromosomes. Pair 1 is metacentric; pairs 2 and 4 are submetacentric; pairs 3, 5, 6 and 7 are acrocentric; and the remaining ones are telocentric. The Z chromosome is submetacentric, and the W is submetacentric in Southern Giant Petrels and metacentric in Cape Petrels. In Black-bellied Storm Petrels (2n = 74), pair 1 is metacentric; pair 3 is submetacentric; pairs 2, 4, 5 and 6 and the remaining ones are telocentric; while the Z is submetacentric, and the W is telocentric. In Wilson's Storm Petrels (2n = 70), pairs 1 and 3 are metacentric; pair 2 is acrocentric; and pairs 4, 5 and 6 are submetacentric; all remaining ones are telocentric. The Z chromosome is metacentric, and the W is submetacentric. All chromosomes except the Z chromosome of Black-bellied Storm Petrels revealed centromeric heterochromatin. The W chromosomes of all species were heterochromatic. We observed a numerical and morphological chromosome homology between the Southern Giant Petrels and the Cape Petrels, but very different phenotypes, while Black-bellied and Wilson's Storm Petrels are phenotypically similar, but their karyotypes differ in chromosome number and morphology.

Key words: cytogenetics, karyotype, chromosomal variability, C-banding, heterochromatin, Antarctic seabirds

INTRODUCTION

Knowledge about the basic biology of class Aves, which includes more than 9000 species, is still incomplete, especially in regard to their genetics and evolution. Argentina and Brazil, for example, have a significant diversity of birds; the karyotype has been described for slightly above 20% (Argentina) and less than 14% (Brazil) of bird species (Santos & Gunski 2006; Cuervo *et al.* 2011). Among Antarctic and sub-Antarctic birds, only four have been cytogenetically analyzed: Antarctic Shag *Leucocarbo bransfieldensis* (2n = 72) (Phalacrocoracidae), Kelp Gull *Larus dominicanus* (2n = 68) (Laridae) and Snowy Sheathbill *Chionis albus* (2n = 76) (Chionidae) (Ledesma *et al.* 2005), all of which were based on conventional staining, as well as Magellanic Penguins *S. magellanicus* (2n = 68) (Spheniscidae) (Ledesma *et al.* 2003), for which differential staining (C-banding) was carried out. The determination of chromosomal characteristics represents an important tool for conservation plans, as well as for phylogenetic and evolutionary studies of this class (Benirschke *et al.* 1980; Bed'Hom *et al.* 2003).

The diploid chromosomal number in birds varies from 42 chromosomes in Coraciiformes (Belterman & De Boer 1990) and 50 chromosomes in Falconiformes to 100 in Rallidae (Gruiformes) and Ramphastidae (Piciformes) and 126 in Bucerotidae (Misra & Srivastava 1976), with a mean of 80 chromosomes (Sasaki *et al.* 1984). In general, birds possess eight pairs of macrochromosomes and 32 pairs of microchromosomes (Tegelström & Ryttman 1981, Tegelström *et al.* 1983).

All birds studied have a conserved sex-chromosome system, with females being heterogametic (ZW) and males homogametic (ZZ) (Belterman & De Boer 1984, Christidis 1990). The Z chromosome is, like the X chromosome in the XY system, larger than the W chromosome and has more genes. Z and W chromosomes differ in the amount of C-banding heterochromatin (Pigozzi & Solari 1999). Heterochromatin is normally associated with repression of transcription and recombination and with late replication; it has a distinctive chromatin structure, shown by the C-band-positive regions (Bickmore 2001).

In this study, we describe for the first time the karyotypes of the following four species of Procellariiformes: Southern Giant *Macronectes giganteus* and Cape Petrels *Daption capense* (Procellariidae); and Black-bellied *Fregetta tropica* and Wilson's *Oceanites oceanicus* Storm Petrels (Hydrobatidae). In addition, we provide the C-banding patterns for their chromosomes.

STUDY AREA AND METHODS

During the Antarctic summers of 1997/98 and 1998/99, feather bulbs or peripheral blood of 34 specimens of Procellariiformes were sampled (Table 1) at Peninsula Potter, King George Island, South Shetland Islands (62°14′S, 58°40′W) and Laurie Island, South Orkney Islands (60°44′S, 44°44′S), Antarctica. The Cape Petrel breeds on Laurie Island, while the other three species of petrels breed at both sampling sites (Hahn *et al*. 1998, Coria *et al*. 2011).

Metaphases were obtained from feather bulbs from juveniles, since juveniles have many feathers undergoing growth and hence intensive cell division, cultured directly for short periods (Giannoni *et al.* 1993). The cellular material of six to eight feather pulps were cultured in a complete RPMI 1640 medium for four hours at 38 °C. This technique was applied for all samples from Cape Petrels and one sample from Southern Giant Petrels.

TABLE 1
Family, scientific and common names, sample size of each sex and sampling location of the studied species

Species	Sample size, sex		Localities
	Male	Female	
Daption capense	6	4	Laurie Island
Macronectes giganteus	6	4	Potter Peninsula
Fregetta tropica	3	5	Laurie Island
Oceanites oceanicus	4	2	Potter Peninsula Laurie Island Potter Peninsula

For the adult individuals, we cultured peripheral blood leukocytes (Moorhead *et al.* 1960), since leukocyte cultures are perhaps the easiest and most convenient method of obtaining chromosome preparations (Biederman & Lin 1982). Heparinized blood (2 mL)

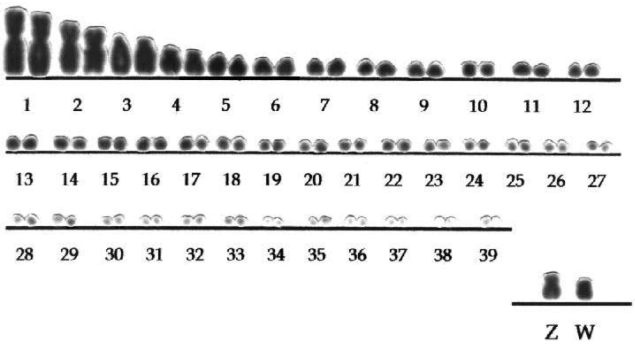
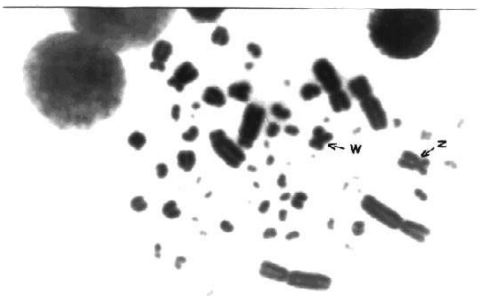


Fig. 2. Metaphase and karyotype of a female Southern Giant Petrel (2n = 80).



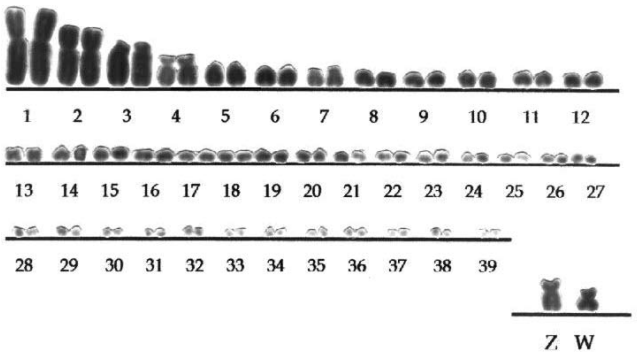


Fig. 1. Metaphase and karyotype of a female Cape Petrel (2n = 80).

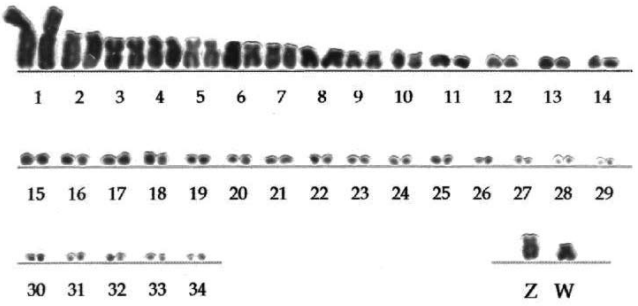


Fig. 3. Metaphase and karyotype of a female Wilson's Storm Petrel (2n = 70).

was collected from the specimens and allowed to separate by gravity. The plasma and white cell band was put into culture flasks containing complete RPMI 1640 medium and incubated at 38 ºC for 72 hours.

Five drops of 0.0016% colchicine were added during the last hour for both cultures. The medium was then removed by centrifuging, and the leukocyte pellet was resuspended in 0.075 mol/L potassium chloride and incubated for 15 min at 38 ºC. The cells were fixed with three consecutive washes of methanol/acetic acid (3:1) fixative. Conventional staining was performed with Giemsa solution in phosphate buffer 0.06 mol/L, pH 6.8 for 15 min. C-banding was performed following the procedure of Sumner (1972).

Biometric analysis (Giannoni *et al.* 1986) was carried out by photographing the 12 best-quality metaphases, i.e., the ones with optimal staining and morphology. The first macrochromosomes with apparent centromere and the sex chromosomes were measured using a millimetrical scale, such that:

$$CR_{\overline{pi}} = \frac{\overline{pi}}{\sum_{i=1}^{12}(\overline{pi}+\overline{qi})+\overline{Z}},\ CR_{\overline{qi}} = \frac{\overline{qi}}{\sum_{i=1}^{12}(\overline{pi}+\overline{qi})+\overline{Z}},$$

$$CR_{(\overline{pi}+\overline{qi})} = \frac{\overline{pi}+\overline{qi}}{\sum_{i=1}^{12}(\overline{pi}+\overline{qi})+\overline{Z}}\,(pi+qi)$$

TABLE 2
Complement of the short (p) and long (q) arms of chromosomes for the four petrels studied

Species		Complement, mean ± standard error						Centromeric index	Arm ratio	Classification
		Relative			Real					
		p	q	p+q	p	q	p+q			
Southern Giant Petrel	1	0.28±0.04	0.40±0.05	0.69±0.08	0.25	0.37	0.62	0.41±0.06	1.43±0.09	Metacentric
	2	0.29±0.03	0.44±0.05	0.73±0.07	0.2	0.3	0.5	0.39±0.02	1.52±0.14	Submetacentric
	3	0.07±0.02	0.29±0.06	0.37±0.02	0.07	0.33	0.4	0.19±0.04	4.21±0.02	Acrocentric
	4	0.17±0.01	0.33±0.02	0.48±0.03	0.1	0.2	0.3	0.34±0.02	1.94±0.21	Submetacentric
	Z	0.16±0.01	0.32±0.03	0.48±0.03	0.1	0.2	0.3	0.34±0.04	1.97±0.38	Submetacentric
	W	0.19±0.01	0.25±0.01	0.44±0.01	0.1	0.12	0.2	0.43±0.04	1.32±0.21	Metacentric
Cape Petrel	1	0.28±0.03	0.42±0.06	0.70±0.09	0.29	0.44	0.73	0.40±0.01	1.49±0.11	Metacentric
	2	0.25±0.02	0.40±0.05	0.65±0.07	0.22	0.36	0.58	0.35±0.09	1.53±0.20	Submetacentric
	3	0.11±0.07	0.48±0.04	0.59±0.10	0.06	0.37	0.43	0.18±0.00	4.43±0.09	Acrocentric
	4	0.18±0.03	0.33±0.03	0.51±0.05	0.1	0.21	0.31	0.35±0.00	1.89±0.04	Submetacentric
	Z	0.01±0.01	0.32±0.02	0.50±0.04	0.09	0.21	0.3	0.32±0.00	1.85±0.04	Submetacentric
	W	0.16±0.01	0.25±0.02	0.41±0.03	0.08	0.13	0.21	0.39±0.00	1.53±0.03	Submetacentric
Wilson's Storm Petrel	1	0.28±0.05	0.41±0.07	0.69±0.12	0.24	0.37	0.61	0.40±0.01	1.41±0.23	Metacentric
	2	0.12±0.02	0.44±0.05	0.57±0.05	0.1	0.3	0.4	0.22±0.03	3.50±0.64	Acrocentric
	3	0.23±0.02	0.28±0.03	0.52±0.04	0.12	0.2	0.3	0.45±0.03	1.21±0.18	Metacentric
	4	0.16±0.03	0.33±0.02	0.50±0.05	0.1	0.2	0.3	0.32±0.06	2.21±0.67	Submetacentric
	5	0.16±0.01	0.32±0.03	0.49±0.04	0.10	0.16	0.26	0.34±0.04	2.01±0.47	Submetacentric
	6	0.15±0.02	0.30±0.02	0.46±0.03	0.06	0.15	0.21	0.33±0.04	2.00±0.40	Submetacentric
	Z	0.19±0.05	0.30±0.03	0.50±0.07	0.1	0.16	0.26	0.37±0.07	1.76±0.59	Submetacentric
	W	0.14±0.01	0.25±0.03	0.40±0.03	0.05	0.11	0.16	0.33±0.12	1.73±0.37	Submetacentric
Black-bellied Storm Petrel	1	0.28±0.06	0.41±0.08	0.69±0.14	0.31	0.46	0.77	0.41±0.01	1.44±0.11	Metacentric
	2	-	0.57±0.06	0.57±0.06	-	0.47	0.47	-	-	Telocentric
	3	0.18±0.01	0.33±0.04	0.52±0.04	0.12	0.23	0.35	0.35±0.03	1.83±0.30	Submetacentric
	4	-	0.48±0.04	0.48±0.04	-	0.31	0.31	-	-	Telocentric
	5	-	0.44±0.44	0.44±0.28	-	0.26	0.26	-	-	Telocentric
	6	-	0.41±0.05	0.41±0.05	-	0.24	0.24	-	-	Telocentric
	Z	0.18±0.01	0.31±0.03	0.50±0.04	0.12	0.2	0.32	0.37±0.02	1.66±0.20	Submetacentric
	W	-	0.32±0.02	0.32±0.02	-	0.07	0.07	-	-	Telocentric

Where:
$CR_{\overline{pi}}$, $CR_{\overline{qi}}$, $CR_{(\overline{pi}+\overline{qi})}$ = relative complement of the short arm, long arm and total, respectively, of the *i*-th chromosome.

$\overline{pi}$ = mean size of the short arm of the *i*-th chromosome in cm.

$\overline{qi}$ = mean size of the long arm of the *i*-th chromosome in cm.

$(\overline{pi}+\overline{qi})$ = mean size of the *i*-th chromosome in cm.

The arm ratio (ar) and the centromeric index (CI) were estimated. The position of the centromere was defined from the ratio of the short arm (q) to the long arm (p): ar = q/p. The size of the short arm multiplied to 100 is divided by the total chromosome size: CI = p×100/ p+q. The chromosomes were ordered from the largest to the smallest, and the karyotypes were classified following the nomenclature proposed by Levan *et al.* (1964), in which chromosomes with CI between 50.0 and 40.1 are referred as metacentric, those with CI between 40.0 and 25.1 as submetacentric, those with CI between 25.0 and 0.01 as acrocentric and those with CI equal to 0 (zero) as telocentric.

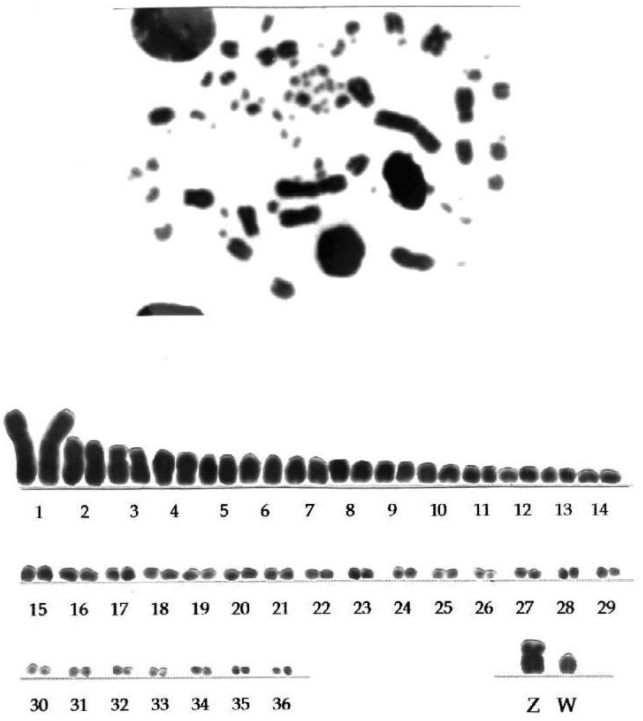


Fig. 4. Metaphase and karyotype of a female Black-bellied Storm Petrel (2n = 74).

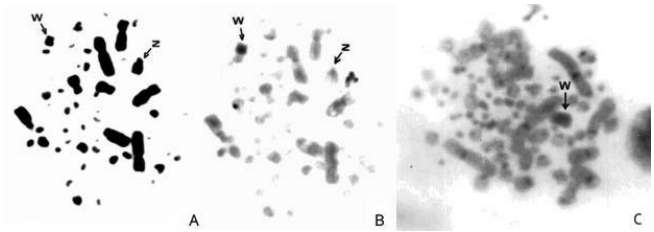


Fig. 5. Metaphase with conventional staining sequence (A) and C-banding (B) of a female exemplar of Cape Petrel (arrows indicate the sex chromosomes). (C) shows centromeric markers (the arrow indicates the totally heterochromatic W chromosome).

RESULTS

The diploid number of Southern Giant and Cape Petrels was 2n = 80, where the first 7 pairs of autosomes and the pair of sex chromosomes were considered macrochromosomes. For Cape Petrels, pair 1 was metacentric, pairs 2 and 4 were submetacentric, pairs 3 and 7 were acrocentric and the remaining pairs were telocentric (Fig. 1). The Z chromosome was submetacentric and equivalent in size to the autosomal pair 4. The W chromosome was submetacentric and equivalent to the autosomal pair 6. Pair 1 of Southern Giant Petrels was metacentric; pairs 2 and 4 were submetacentric; and pairs 3, 5, 6 and 7 were acrocentric. The remaining chromosomes were all telocentric microchromosomes (Fig. 2). The Z chromosome was submetacentric, equivalent in size to the autosomal pair 4. The W chromosome was metacentric, equivalent in size to the autosomal pair 8.

In Wilson's Storm Petrels (2n = 70), the first nine pairs and the sex pair were macrochromosomes (Fig. 3). Pairs 1 and 3 were metacentric; pair 2 was acrocentric; and pairs 4, 5 and 6 were submetacentric (Table 2). Pairs 7, 8 and 9 were apparently acrocentric. The remaining 25 chromosome pairs were considered telocentric microchromosomes,

Fig. 6. Metaphase with conventional staining sequence (Giemsa, left) and C-banding (right) of a female exemplar of Southern Giant Petrel (arrows indicate the sex chromosomes).

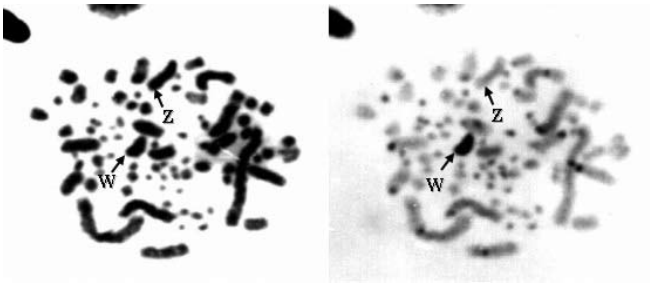


Fig. 7. Metaphase with conventional staining sequence (Giemsa, left) and C-banding (right) of a female exemplar of Black-bellied Storm Petrel (arrows indicate the sex chromosomes).

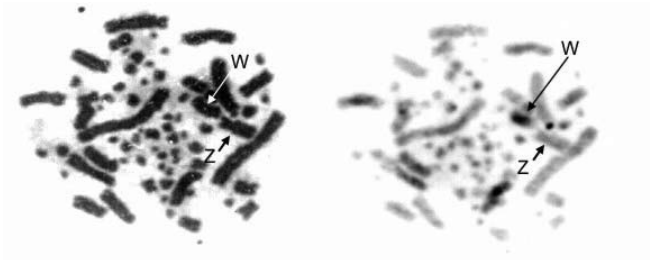


Fig. 8. Metaphase with conventional staining sequence (Giemsa, left) and C-banding (right) of a female exemplar of Wilson's Storm Petrel (arrows indicate the sex chromosomes).

except pair 10, which was metacentric. The sex chromosome Z was submetacentric and equivalent in size to pair 5. The W chromosome was submetacentric and equivalent in size to pair 9.

In Black-bellied Storm Petrels (2n = 74), pair 1 was metacentric; pairs 2, 4, 5 and 6 were telocentric; and pair 3 was submetacentric. All of the remaining chromosomes were telocentric (Table 2). The sex chromosome Z was submetacentric and equivalent in size to pair 3. The W chromosome was telocentric and equivalent in size to pair 10 (Fig. 4).

The sequential Giemsa–C-banding analysis allowed the identification of the W chromosome, which was entirely heterochromatic in all four species (Figs. 5, 6, 7 and 8). The remaining chromosomes presented positive marks in the centromeric region, including the Z chromosome, except in Black-bellied Storm Petrels (Fig. 7). However, in Wilson's Storm Petrels, few chromosomes reacted positively to the salt treatment (Fig. 8). The microchromosomes of Southern Giant Petrels showed no C-banding markings (Fig. 6).

DISCUSSION

This work presents the first cytogenetic analysis of the Antarctic seabirds Cape, Southern Giant, Black-bellied Storm and Wilson's Storm Petrels. Southern Giant Petrels and Cape Petrels were numerically and morphologically homologous, although they are phenotypically very different. Southern Giant and Cape Petrels homology also occurs in Tinamiformes (Lucca 1985, Garnero & Gunski 2000, Garnero *et al.* 2006), Rheiformes (Takagi *et al.* 1972, Boer 1980, Ansari *et al.* 1988, Liotta & Gunski 1998) and Anseriformes (Aguiar 1968), in which the high chromosomal uniformity observed suggests that genetic mutations predominated during lineage differentiation. Sphenisciformes and Procellariiformes are thought to have a common ancestor (Livezey & Zusi 2006, 2007; MacCormack *et al*. in press), since the same karyotypic uniformity is observed in both orders, but with a high phenotypic similarity in penguins (Jensen 1973, Takagi & Sasaki 1974, Ledesma *et al.* 2003, 2005).

Chromosomal variability associated with phenotypic variability occurs frequently. However, the opposite situation is seen in the family Hydrobatidae, in which Black-bellied Storm Petrels (2n = 74) and Wilson's Storm Petrels (2n = 70) have different chromosomal numbers and morphology, although they are phenotypically very similar. In Wilson's Storm Petrels, a trend toward reduced chromosomal number and increased number of biarmed chromosomes was observed (Table 2), likely due to Robertsonian translocations (centric-fusion translocations), as seen in other species (Tegelström & Ryttman 1981; Lucca 1985; de Oliveira *et al.* 2010). The great difference between these two species was in the morphology of the largest chromosomes and the W chromosome. In Wilson's Storm Petrels, pair 2 was acrocentric and pairs 4, 5, 6 and W submetacentric, but in Black-bellied Storm Petrels all of these chromosomes were telocentric. Comparable differences were observed in the family Tyrannidae, in which some phenotypically similar species also showed karyotypic differences in the largest chromosomes of the complement (Gunski *et al.* 2000).

As in the majority of the species, constitutive heterochromatin was found in the centromeric regions, except in the Z chromosome of Black-bellied Storm Petrels, chromosomes of Wilson's Storm Petrels (which failed to react positively to the salt treatment) and microchromosomes of Southern Giant Petrels. The W sex chromosome holds many highly repeated sequences, and this could explain why we observed totally positive C-banding in all species.

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