Oceanic respite for wandering albatrosses

Birds taking time off from breeding head for their favourite long-haul destinations.

What oceanic seabirds do outside their breeding periods is something of a mystery, although altogether these 'sabbaticals' add up to more than half of their lifetime and are probably a key feature of their life history. Here we use geolocation systems based on light-intensity measurements to show that during these periods wandering albatrosses (*Diomedea exulans*) leave the foraging grounds that they frequent while breeding for specific, individual oceanic sectors and spend the rest of the year there — each bird probably returns to the same area throughout its life. This discovery of individual home-range preferences outside the breeding season has important implications for the conservation of albatrosses threatened by the development of longline fisheries.

On 26 October 1999, a wandering albatross of at least 50 years old was found dead on a beach in New South Wales, Australia (Fig. 1). A ring identified the bird as coming from the Crozet Islands, 7,900 km away in the Indian Ocean; this bird was originally banded in September 1960 as an adult (over 10 years old) a few kilometres from the site of its death. This recovery of a bird after 40 years, together with the successive recapture every second year of several individuals from Crozet at the same site[1], suggests that wandering albatrosses, which breed every second year, may return routinely throughout their lifetime to a particular place during the non-breeding year.

It has been assumed that these birds wander aimlessly during their biennial sabbatical year, circumnavigating the Southern Ocean by riding the wind[2,3], visiting places like the waters of New South Wales en route. The question of where these birds spend half of their life is important (Fig. 2), not only because this species may potentially exploit almost any area in the Southern Ocean[4], but also because wandering albatrosses, like many other albatrosses and petrels, are threatened by longline fisheries in the Southern Ocean. They are killed in tens of thousands while scavenging for baited hooks and from drowning when lines are set[5]. Any plan to remedy this shocking loss will depend on establishing the extent of overlap in the areas used by both albatrosses and fisheries.

Using satellite telemetry to study the movements of seabirds over a complete year is difficult and costly. Instead, we determined bird position using loggers weighing 20 g that record light intensity and are attached to a ring on the bird's leg. We were able to estimate the local time of dawn and dusk, determined by light thresholds, with respect to Greenwich Mean Time using a precise internal clock: after consideration of the date, the day length could be used to determine the latitude, and the longitude was determined from local time of noon[6–8].

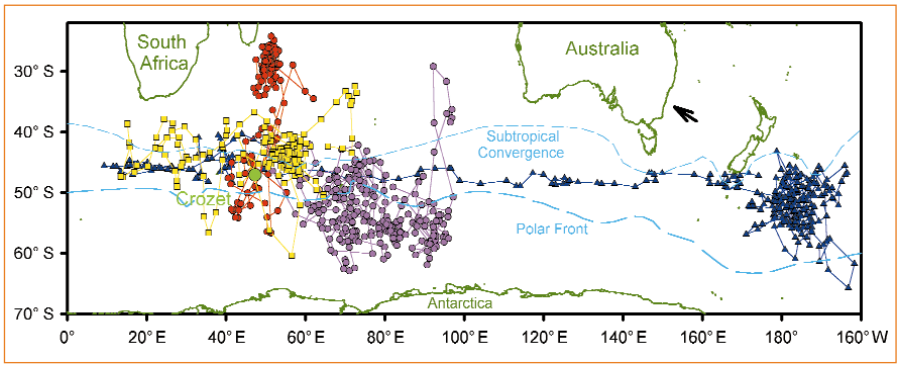

Figure 1 Oceanic sectors used by two male (mauve dots and blue triangles) and two female (red dots and yellow squares) wandering albatrosses, as studied by geolocation. The dots indicate two partners of the same pair. Two locations are estimated for each bird per 24-h cycle. Arrow indicates the recovery site of ringed birds off New South Wales, Australia; green circle, the Crozet Islands.

We fitted nine wandering albatrosses on the Crozet Islands with loggers during February–March in 1995 and 1996, just after a failed breeding attempt and before their departure from the breeding grounds for a year. This pattern of absence from the breeding grounds is similar to that adopted by birds that complete a successful breeding season in December[1]. All tagged birds returned one year later with the unit intact, although only four devices provided light measurements over 6 months or more.

After leaving the island, the four birds (two males and two females) flew to a specific ocean sector where they overwintered, returning to their breeding place in late December. These sectors ranged from tropical and subtropical waters (the females) to sub-Antarctic and Antarctic waters (the males) at distances 1,500–8,500 km away from Crozet. In each pair, the male spent the winter just north of the pack ice in Antarctic waters, whereas the female stayed south of Madagascar.

To our knowledge, this is the first time that the wintering zones of a pelagic seabird have been studied remotely. Our results provide surprising evidence that wandering albatrosses do not wander around the Southern Ocean during their non-breeding year. After spending a year covering an estimated 150,000 km foraging around colonies to hatch and raise their single chick[4,9], wandering albatrosses take a year off. The vast distances covered during breeding increase the probability of encountering dispersed prey while effectively using the wind to reduce flight costs[9]. During the sabbatical year, the birds have lower energy requirements and are not restricted to a central breeding place.

But even with this new freedom, the birds confine their movements to a preferred sector, not only because of their reduced energy requirements but maybe also because at this time they undergo a partial moult of their flight feathers. Moulting probably reduces flight efficiency and therefore increases flight costs. Also, by moving away to a distant ocean sector far from the breeding grounds, the competition between the element of the population in its breeding year and the other in its sabbatical year is reduced. Information from banding indicates that birds use the same wintering zone from year to year, probably throughout their lifetime. The wintering grounds favoured by each individual in the open ocean may be visited repeatedly because the birds learn over years the location of profitable feeding zones.

Our results have important implications

Figure 2 The wandering albatross spends more than 95% of its life in the open ocean, ten years of this as as an immature bird, but also half of its mature life during its non-breeding years. Birds move to specific oceanic areas far from their breeding grounds; area preferences appear to last a lifetime.

for the conservation of this threatened species[10]. During the breeding season, females favour the subtropical waters north of Crozet where tuna fisheries are located, whereas males prefer the colder waters at higher latitudes where fisheries for toothfish have been developed[11]. Birds spending their sabbatical year in sectors where longline fisheries occur are at risk of being killed. This bizarre selection pressure, dependent perhaps on the whims of juvenile choice, means that only those wintering in zones without fisheries will survive in the long term. We now need to find out what factors lead to selection of sabbatical areas in the first place. In the meantime, we have to accept that even during their sabbaticals adult birds are unlikely to change their habits to avoid the dangers from fisheries.

Henri Weimerskirch*†, Rory P. Wilson‡
*CEBC-CNRS, 79360 Villiers en Bois, France
e-mail: henriw@cebc.cnrs.fr
†*Institut Français pour la Recherche et la Technologie Polaire, 29280 Plouzane, France*
‡*Institüt für Meereskunde, D-24105 Kiel, Germany*

1. Weimerskirch, H., Jouventin, P., Mougin, J. L., Stahl, J. C. & Van Beveren, M. *Emu* **85,** 22–23 (1985).
2. Murphy, R. C. *Oceanic Birds of South America* (Macmillan, New York, 1936).
3. Nicholls, D. G., Murray, M. D., Butcher, E. & Moors, P. *Emu* **97,** 240–244 (1997).
4. Jouventin, P. & Weimerskirch, H. *Nature* **343,** 746–748 (1990).
5. Brothers, N. P. *Biol. Conserv.* **55,** 255–268 (1991).
6. Wilson, R. P., Ducamp, J. J., Rees, G., Culik, B. M. & Niekamp, K. in *Wildlife Telemetry: Remote Monitoring and Tracking of Animals* (eds Priede, I. M. & Swift, S. M) 131–134 (Horward, Chichester, 1992).
7. Hill, R. D. in *Elephant Seals: Population Ecology, Behavior, and Physiology* (eds Le Boeuf, B. J. & Laws, R. M.) 227–236 (Univ. California Press, Berkeley, 1994).
8. Welch, D. W. & Eveson, J. P. *Can. J. Fish. Aquat. Sci.* **56,** 1317–1327 (1999).
9. Weimerskirch, H., Doncaster, P. & Cuénot-Chaillet, F. *Proc. R. Soc. Lond. B* **255,** 91–97 (1994).
10. Robertson, G. & Gales, R. *Albatross, Biology and Conservation* (Beatty, Chipping Norton, Australia, 1993).
11. Weimerskirch, H., Brothers, N. P. & Jouventin, P. *Biol. Conserv.* **79,** 257–270 (1997).

Plant biotechnology

Caffeine synthase gene from tea leaves

Caffeine synthase is an enzyme that catalyses the final two steps in the caffeine biosynthesis pathway. We have cloned the gene encoding caffeine synthase from young leaves of tea (*Camellia sinensis*), opening up the possibility of creating tea and coffee (*Coffea arabica*) plants that are naturally deficient in caffeine. Consumers concerned about the possible adverse effects of caffeine consumption will welcome this development towards caffeine-free drinks that retain their flavour.

The increasing demand for decaffeinated coffee and tea has resulted from the occasional side effects of caffeine, which include palpitations, gastrointestinal disturbances, anxiety, tremor, increased blood pressure and insomnia[1,2]. At present, the decaffeination process depends on supercritical fluid extraction with carbon dioxide to avoid introducing toxic residues from extraction solvents. However, this operation is expensive and, to discerning customers, flavours and aromas are lost.

There are some *Coffea* and *Camellia* species that produce low levels of caffeine, but these are not readily available for commercial use. Although it might be feasible to develop a breeding programme, it could take 20 years or so to establish and stabilize the desired traits[3]. The large-scale production of transgenic caffeine-deficient *Camellia sinensis* and *Coffea arabica* plants may thus be a more practical proposition[4], but first some information is needed about the genes controlling key conversions in the biosynthesis of caffeine.

Caffeine is synthesized from purine nucleotides. The two final steps of the pathway, in which two methyl groups are added successively to 7-methylxanthine to produce theobromine and then caffeine, are catalysed by caffeine synthase, a bifunctional enzyme comprising two *S*-adenosylmethionine-dependent *N*-methyltransferase activities[3]. It has been hard to purify and isolate caffeine synthase and other enzymes of this pathway because they are extremely labile, but the amino-terminal sequence of caffeine synthase from young tea leaves has now been reported[5].

We used the RACE (rapid amplification of complementary DNA ends) technique with degenerate gene-specific primers based on the amino-terminal sequence of caffeine synthase to obtain a 1.31-kilobase sequence of cDNA. The 5′ untranslated sequence of the cDNA fragment was isolated by 5′ RACE. The isolated cDNA, termed TCS1 (GenBank accession no. AB031280), consists of 1,438 base pairs and encodes a protein of 369 amino acids. The deduced amino-acid sequence of TCS1 shares a small amount of sequence similarity with other *N*-, *S*- and *O*-methyltransferases from plants and microorganisms, but considerably more with the salicylic acid *O*-methyltransferase[6] (41.2%).

To determine whether our cDNA encoded an active caffeine synthase enzyme, we expressed TCS1 in *Escherichia coli* and incubated lysates of the bacterial cells with a variety of xanthine substrates in the presence of *S*-adenosylmethionine as methyl donor. We found that the substrate specificity of the recombinant enzyme was very similar to that of the native enzyme purified from young tea leaves (Table 1), with the recombinant enzyme mainly catalysing 3-*N*-methylation and 1-*N*-methylation of the purine ring of mono- and dimethylxanthines. There was no 7-*N*-methylation activity when xanthosine was the methyl acceptor. These results indicate that TCS1 encodes caffeine synthase.

The cloning of the caffeine synthase gene is an important advance towards the development of transgenic caffeine-deficient *Camellia sinensis* and *Coffea arabica* plants through antisense messenger RNA technology or by gene silencing. It is possible that the health benefits of tea[7–11], whose catechins and related polyphenols[7–10] are thought to help protect against heart disease, may be enhanced without the potentially hypertensive effects of caffeine[12,13].

Misako Kato*, Kouichi Mizuno†, Alan Crozier‡, Tatsuhito Fujimura†, Hiroshi Ashihara*
**Department of Biology, Faculty of Science, Ochanomizu University, Otsuka, Bunkyo-ku, Tokyo 112-8610, Japan*
†*Institute of Agricultural and Forest Engineering, University of Tsukuba, Ibaraki 305-8572, Japan*
‡*Plant Products and Human Nutrition Group, Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK*
e-mail: a.crozier@bio.gla.ac.uk

1. Chou, T. M. & Benowitz, N. L. *Comp. Biochem. Physiol.* **109C,** 173–189 (1994).
2. Nurminen, M.-L., Niittynen, L., Korpela, R. & Vapaatalo, H.

Table 1 Substrate specificity of recombinant and native caffeine synthase

Substrate	*Methylated product*	*N-methylation position*	*Recombinant CS*	*Native CS**
Monomethylxanthines				
7-Methylxanthine	Theobromine	3	100†	100
3-Methylxanthine	Theophylline	1	1.0	17.6
1-Methylxanthine	Theophylline	3	12.3	4.2
Dimethylxanthines				
Theobromine	Caffeine	1	18.5	26.8
Theophylline	Caffeine	7	<0.1	<0.1
Paraxanthine	Caffeine	3	230	210
Others				
Xanthosine	7-Methylxanthosine	7	Not detected	Not detected

The full-length coding region for the tea-leaf caffeine synthase (CS) protein was ligated into pET23d plasmids and the resultant expression vector introduced into *E. coli* (BL21). Recombinant CS protein was extracted by sonication of the transformed cells in 50 mM Tris-HCl (pH 7.5) containing 1 mM EDTA-Na_2 and 0.1 M NaCl. The substrate specificity of recombinant CS was determined according to ref. 5. CS activity is expressed as a percentage of the activity on 7-methylxanthine.
*Caffeine synthase activity of the recombinant enzyme with 7-methylxanthine (100%) was 5.4 pkat mg^{-1} protein; values represent the average of duplicate samples.
†Taken from ref. 5.