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Appendix 1

Methods

Antioxidant and uric acid analysis

Trolox-equivalent antioxidant capacity (TEAC) of serum was measured following Cohen et al. (2007). The assay uses a chromogenic free radical, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, Sigma), activated by H_2O_2 . As the free radical is activated, the solution shows a linear increase in absorbance; any micromolecular antioxidants in the sample delay the start of this increase by quenching the free radical as it is activated. When the antioxidants are exhausted, absorbance increase begins. Antioxidant capacity is thus quantified by measuring the delay in start of increase relative to Trolox (Aldrich), a water-soluble vitamin E analogue used as the standard. All measurements were conducted on a spectrophotometer. Coefficient of variation for individual samples is generally around 7% across assays. This assay gives a functional measure of antioxidant capacity, i.e. it reveals how effective the sample is at quenching free radicals. It does not measure antioxidant contributions of proteins or enzymes, and says nothing about antioxidant activity in tissues. Primary antioxidants contributing to the assay include uric acid, vitamins E and C, carotenoids, phenolics and bilirubin (Miller et al. 1993). Uric acid concentration was quantified with a spectrophotometric kit based on uricase and a chromogen. The samples can be run

alongside the antioxidant samples in the same microplate. Average coefficient of variation is 5% across assays.

Carotenoid and vitamin E analysis

Lipids were extracted by sequentially adding 100 μ l ethanol and 100 μ l tert-butyl methyl ether to 5–10 μ l serum, vortexing, and centrifuging for 15 s at 10000 RPM. We transferred the supernatant to a fresh tube, evaporated the solvent to dryness under a stream of nitrogen, and redissolved the extract in 200 μ l of 42:42:16 (v/v/v) methanol:acetonitrile:dichloromethane. Carotenoids and vitamin E were subsequently analyzed using high-performance liquid chromatography (HPLC), following previously published methods (McGraw and Parker 2006). Pigment extracts were injected into a HPLC system fitted with a carotenoid 5.0 μ m column (4.6 \times 250 mm) and a built-in column heater set at 30°C. We used a three-step gradient solvent system to analyze both xanthophylls and carotenes in a single run, at a constant flow rate of 1.2 ml min⁻¹: first, isocratic elution with 42:42:16 (v/v/v) methanol:acetonitrile:dichloromethane for 11 min, followed by a linear gradient up to 42:23:35 (v/v/v) methanol:acetonitrile: dichloromethane through 21 min, held isocratically at this condition until 30 min, and finishing with a return to the initial isocratic condition from 30–48 min. Data were collected from 250–600 nm using a photodiode array detector. We identified molecules by comparing their respective retention times and absorbance maxima (λ_{max}) to those of pure standards.

Appendix 2

Discussion

Leach's storm-petrels – variation by time-of-day

Sampling of Leach's storm-petrels was conducted both during the day, when birds are inactive in burrows, and at night, when birds are active. Nearly all antioxidant parameters showed markedly higher values at night. Over many years, attempts to catch birds at night in this way almost never resulted in capture of individuals banded in burrows, suggesting that the night-caught birds are mostly pre-breeders (Huntington unpubl.). Differences between day- and night-caught birds could thus be due to activity level, time-of-day, age, breeding status, or some combination thereof. The differences are larger than many interspecific differences observed, and the daytime values are generally in line with those for waved albatrosses *Diomedea irrorata* and Nazca boobies *Sula granti*, which are not nocturnal – all three have TEAC, uric acid, and carotenoid levels lower than most land birds, though vitamin E is higher (Table A3). In chickens *Gallus domesticus*, TEAC apparently responds to cycles of melatonin, a hormone involved in circadian rhythms and an antioxidant itself (Albarrán et al. 2001). As in Leach's storm-petrels, TEAC is higher at night, but chickens are diurnal, whereas Leach's storm-petrels are nocturnally active during breeding. Here, in most cases we did not know on which day of the 44-day incubation period day-caught birds were sampled, but there were no differences in TEAC by day of incuba-

tion in a separate dataset (Mauck unpubl.). This seems to contrast with incubating (fasting) common eiders *Somateria mollissima*, in which uric acid increases as protein is catabolized (Hollmén et al. 2001).

Savannah sparrows – breeding status issues

Unlike in Leach's storm-petrels, in Savannah sparrows TEAC and uric acid levels showed no association with reproductive rate or age, but did show a positive association with BMI. Age was unassociated with any antioxidant measure. Age was also unassociated with reproductive rate in this data set, though older birds have previously been shown to have higher reproductive success by a number of measures (Wheelwright and Schultz 1994). We were not able to control precisely for breeding status here, but it appears that only one individual may have been laying when sampled; the rest were known or presumed to be incubating or feeding nestlings. Antioxidant levels do vary with season in some species (Cohen unpubl.), but they did not differ between incubating and feeding birds in our sample, and there is reason to suspect that such differences would not be great. The largest seasonal fluctuations in hormones tend to be around the courtship and laying period rather than between incubating and feeding, though there is considerable variation across different species and different hormones (Wingfield and Farner 1993). Also, in many species the largest shift in diet occurs after parents stop feeding young, not between incubating and feeding; observations suggest that this is also true in Savannah sparrows, which rely heavily on invertebrates throughout the breeding season (Wheelwright unpubl.).

Table A1. Mean and standard error for measures in Leach's storm-petrels.

	Day					Night					t-test
	n	mean	SE	min	max	n	mean	SE	min	max	p-value
TEAC	42	0.35	0.01	0.23	0.51	13*	1.30	0.15	0.59	2.53	<0.0001
Uric acid (mg dl ⁻¹)	42	3.06	0.19	0.96	5.67	14	21.87	2.30	9.09	41.66	<0.0001
TEAC-UA residual	42	-0.04	0.01	-0.20	0.13	13*	0.21	0.04	-0.21	0.41	<0.0001
Vitamin E (abs. units)	27	2922	128	1874	4142	10	4629	357	3378	7077	0.0008
Lutein (µg ml ⁻¹)	27	0.09	0.02	0	0.58	10	0.22	0.06	0.04	0.66	0.08
Zeaxanthin (µg ml ⁻¹)	27	2.19	0.22	0	5.45	10	3.72	0.26	2.44	5.32	0.0005
Total carotenoids (µg ml ⁻¹)	27	2.28	0.24	0	5.68	10	3.94	0.31	2.48	5.97	0.0006
Tarsus (mm)	23	24.56	0.10	23.6	25.5	14	24.85	0.19	23.8	26.5	0.15
Mass (g)	0					14	43.7	0.63	40.6	48.3	
Breeding age (years)	41	7.78	1.38	0	28	0					
Hatch rate	18	0.81	0.06	0	1	0					

*one outlier was excluded.

Table A2. Mean and standard error for measures in Savannah sparrows.

	Males					Females					t-test
	n	mean	SE	min	max	n	mean	SE	min	max	p-value
TEAC	17	2.80	0.33	1.47	7.20	24	2.96	0.23	1.38	6.52	0.55
Uric Acid (mg dl ⁻¹)	16	44.3	3.41	20.9	74.2	24	50.3	3.20	21.3	76.4	0.27
Vitamin E (abs. units)	15	2625	140	1938	3620	22	2280	160	1375	4210	0.14
Lutein (µg ml ⁻¹)	15	22.6	2.82	9.01	40.3	22	21.3	2.28	5.05	41.7	0.74
Zeaxanthin (µg ml ⁻¹)	15	3.71	0.25	1.98	5.65	22	2.83	0.25	0.88	5.15	0.02
β-cryptoxanthin (µg ml ⁻¹)	15	2.25	0.46	0.29	5.39	22	1.30	0.36	0	7.55	0.02
β-carotene (µg ml ⁻¹)	15	0.17	0.07	0	0.74	22	0.21	0.07	0	1.10	0.71
α-cryptoxanthin (µg ml ⁻¹)	15	2.16	0.86	0	13.3	22	1.05	0.29	0	4.77	0.08
Total carotenoids (µg ml ⁻¹)	15	30.9	2.58	17.3	47.1	22	26.7	2.37	7.07	48.5	0.25
Fledglings year ⁻¹	3	4.83	0.50	4	5	8	4.8	0.34	4	6	0.96
Age (years)	17	1.24	0.14	1	3	24	1.71	0.35	1	5	0.11
Mass (g)	17	20.3	0.29	18.3	22.0	24	19.2	0.25	17.0	21.4	0.006
Tarsus (mm)	17	21.3	0.16	20.1	22.6	24	20.8	0.12	19.5	21.7	0.01
BMI (100×mass/tarsus ²)	17	4.47	0.06	4.02	5.04	24	4.45	0.07	3.90	5.15	0.82

Table A3. Mean antioxidant levels of our study species compared to those of a selected range of other species.

Common name	Genus	species	TEAC	Uric acid (mg dl ⁻¹)	Vitamin E (abs. units)	Lutein (µg ml ⁻¹)	Zeaxanthin (µg ml ⁻¹)	β-cryptoxanthin (µg ml ⁻¹)	β-carotene (µg ml ⁻¹)	Total carotenoids (µg ml ⁻¹)
Waved albatross	<i>Diomedea</i>	<i>irrorata</i>	0.21	2.5	2869	0.26	2.13	0.00	0.00	2.40
Swainson's thrush	<i>Catharus</i>	<i>ustulatus</i>	0.27	4.5	2900	9.52	0.33	0.00	0.00	9.85
Blue-crowned motmot	<i>Momotus</i>	<i>momota</i>	0.49	10.8	2217	4.01	0.00	0.00	0.00	4.02
Golden-collared manakin	<i>Manacus</i>	<i>vitellinus</i>	0.62	9.2	1198	34.50	3.01	0.11	0.91	38.53
Leach's storm petrel	<i>Oceanodroma</i>	<i>leucorhoa</i>	0.64	7.8	3383	0.13	2.60	0.00	0.00	2.73
Ruddy ground dove	<i>Columbina</i>	<i>talpacoti</i>	0.66	8.5	983	3.01	0.98	0.02	0.00	4.01
Nazca booby	<i>Sula</i>	<i>granti</i>	0.73	11.1	3265	0.02	0.67	0.00	0.00	0.69
Social flycatcher	<i>Myiozetetes</i>	<i>similis</i>	0.74	11.0	2023	71.29	7.65	0.00	2.16	81.09
Flame-rumped tanager	<i>Ramphocelus</i>	<i>flammigerus</i>	0.74	2.2	1940	2.05	10.59	0.24	0.00	12.88
Cedar waxwing	<i>Bombycilla</i>	<i>cedrorum</i>	0.75	7.3	1731	7.54	5.81	0.19	0.15	13.91
Killdeer	<i>Charadrius</i>	<i>vociferus</i>	1.11	23.5	3403	3.35	0.76	0.52	0.03	4.66
American robin	<i>Turdus</i>	<i>migratorius</i>	1.29	19.5	1585	2.27	1.66	0.00	0.03	3.96
Variable seedeater	<i>Sporophila</i>	<i>americana</i>	1.31	18.1	971	15.59	4.21	0.00	0.14	19.94
Florida scrub-jay	<i>Aphelocoma</i>	<i>coerulescens</i>	1.46	25.6	4089	1.01	0.80	0.00	0.00	1.81
Spotted antbird	<i>Hylophylax</i>	<i>naevioides</i>	1.69	33.2	1892	3.57	0.12	0.00	0.00	3.69
Downy woodpecker	<i>Picoides</i>	<i>pubescens</i>	1.81	40.0	1650	11.77	0.07	0.00	0.00	11.83
Field sparrow	<i>Spizella</i>	<i>pusilla</i>	2.04	36.1	1552	25.47	1.76	0.00	0.45	27.69
Song sparrow	<i>Melospiza</i>	<i>melodia</i>	2.15	35.1	1820	19.46	2.60	0.80	0.44	23.30
Rose-breasted grosbeak	Pheucticus	ludovicianus	2.20	35.5	5287	9.89	0.83	0.00	0.00	10.72
Eastern phoebe	<i>Sayornis</i>	<i>phoebe</i>	2.24	38.6	2678	12.08	1.40	0.00	0.00	13.48
Chipping sparrow	<i>Spizella</i>	<i>passerina</i>	2.38	44.8	1787	9.08	1.18	0.24	0.49	10.97
Tree swallow	<i>Tachycineta</i>	<i>bicolor</i>	2.67	50.4	6687	1.05	1.85	2.28	0.00	5.44
Savannah sparrow	<i>Passerculus</i>	<i>sanduichensis</i>	2.88	48.0	2414	21.34	3.17	1.69	0.18	28.27
Yellow warbler	<i>Dendroica</i>	<i>petechia</i>	3.21	54.5	2645	57.74	11.04	2.89	1.35	73.02
Indigo bunting	<i>Passerina</i>	<i>cyanea</i>	5.14	72.6	600	24.67	1.27	0.00	0.00	25.95
Rosy thrush-tanager	<i>Rhodinocichla</i>	<i>rosea</i>	5.18	50.1	1610	0.95	0.00	0.00	0.00	0.95
Black-capped chickadee	<i>Parus</i>	<i>atricapilla</i>	5.23	70.2	3296	5.71	0.28	0.00	0.00	5.99

Data taken from Cohen (2007) to include species both closely and distantly related to our study species, and to include those with the highest and lowest levels of each antioxidant. α-cryptoxanthin was present only in Savannah sparrows (mean = 1.50 µg ml⁻¹) and tree swallows (mean = 0.26 µg ml⁻¹).

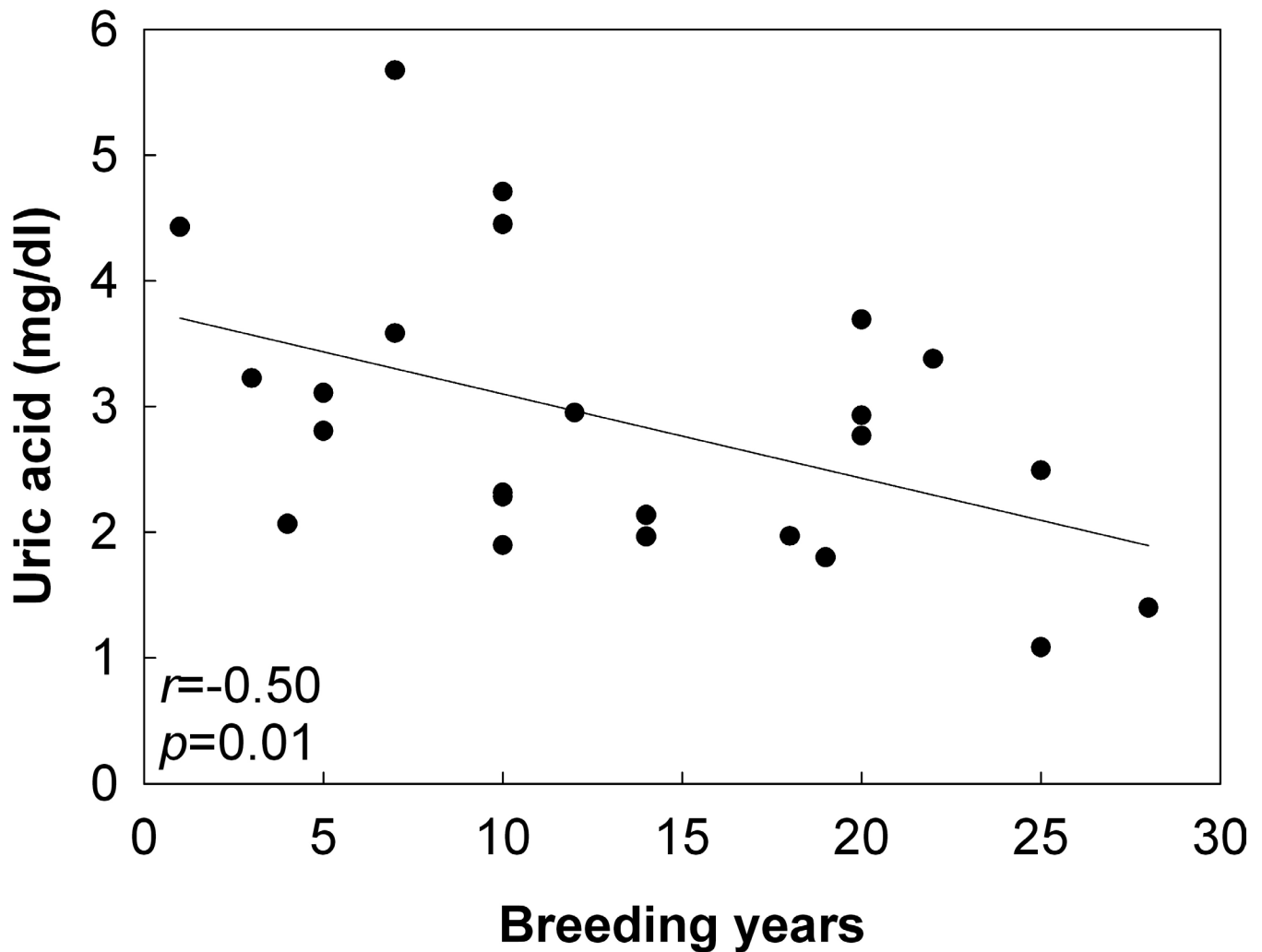


Figure A1. Relationship between uric acid and breeding age in Leach's storm-petrels. Breeding generally starts at age 4–6.

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