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Effects of carotenoid supply on escape flight responses in zebra finches, *Taeniopygia guttata*

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Dietary carotenoids perform several important roles in animals including as antioxidants, immunostimulants and pigments responsible for sexual signals. However, carotenoids may be in limited supply because of foraging constraints and/or physiological trade-offs in their utilization. Studies of birds and fish have shown that females frequently prefer to mate with carotenoid-rich males, which may serve to ensure the acquisition of a healthier mate. Evidence suggests that antioxidant and immune defences can be constrained by carotenoid availability, but we still lack information about the functional consequences of such carotenoid limitation for health, or any measure of physiological performance at the behavioural level. We tested whether carotenoid availability influenced escape flight responses in captive male zebra finches. Birds were fed a control or carotenoid-supplemented diet for 8 weeks, before measurements of escape flight responses from a release chamber after a startle stimulus. Carotenoid supplementation enhanced flight performance: carotenoid-supplemented birds had shorter flight times than controls. In addition, compared to controls, carotenoid-supplemented birds less often required a repeat startle stimulus to elicit escape flight, and emerged sooner from the release chamber after a startle stimulus. Such effects of carotenoids could be ecologically important, since flight take-off performance is thought to be an important determinant of predator evasion and foraging, and thus of survival probability and the capacity to provide parental care. We discuss various putative physiological mechanisms to explain how carotenoids may influence flight behaviour and performance.

Carotenoid pigments are biologically active, fat-soluble compounds that act as antioxidants and stimulate various aspects of immune function (Chew 1996). They are also responsible for red, orange and yellow secondary sexual coloration in many animal species (reviewed in Møller et al. 2000). However, carotenoid availability may be physiologically limiting. This is because animals cannot synthesize carotenoids de novo, so must obtain them through their diet (Goodwin 1984), but also because there may be trade-offs in the allocation of carotenoids between competing somatic demands such as sexual display and antioxidant and immune functions (Lozano 1994; von Schantz et al. 1999).

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Females in many bird species frequently prefer to mate with carotenoid-rich males (reviewed in Andersson 1994). One potential explanation for such mate preference is that carotenoid-rich males are healthier individuals that can provide high-quality direct benefits such as resource-defending ability and parental care (Lozano 1994; von Schantz et al. 1999). Supplemental feeding studies of captive zebra finches suggest that carotenoid availability is limiting for sexual attractiveness (Blount et al. 2003b), immune responses after experimental challenge (Blount et al. 2003b; McGraw & Ardia 2003) and resistance to oxidative damage (Alonso-Alvarez et al. 2004). However, we still lack information about the functional consequences of such carotenoid limitation for health or for any measure of physiological performance at the behavioural level. There is correlational evidence that males with more intense carotenoid-based coloration have superior foraging success for carotenoids (Hill et al. 2002) and work harder to provision mates or nestlings (e.g. Hill 1991; Linville et al. 1998; Senar et al. 2002). For example, in a cross-fostering experiment using blue tits, *Cyanistes caeruleus*,

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Senar et al. (2002) showed that chick growth was related to the plumage yellowness of the foster father but not to that of the genetic parents. However, no study has been able to clarify whether a greater capacity for work in carotenoid-rich males reflects condition dependence in exercise performance as influenced by carotenoids or alternatively is genetically determined. One possibility is that carotenoid availability may be limiting for exercise performance, because of the role of antioxidants in protecting muscles against oxidative damage (reviewed in Powers et al. 2004). However, we are not aware of any study that has manipulated the availability of carotenoids and then measured the effects on physical exercise.

In a study of male zebra finches we manipulated carotenoid availability by providing birds with either a standard seed diet including some carotenoids (control group) or the same diet supplemented with additional carotenoids. We then assessed the escape flight responses of individuals after a startle stimulus. Take-off from the ground is physiologically challenging, requiring high burst power output, and is thought to be an important determinant of predator evasion and foraging performance (Witter & Cuthill 1993; Metcalfe & Ure 1995; Kullberg et al. 1996; Veasey et al. 1998). We predicted that carotenoid supplementation would enhance escape flight performance.

METHODS

Birds and Maintenance

During May–June 2003 in a matched-pairs experimental design, we randomly allocated 11 pairs of zebra finch brothers to receive either plain drinking water (controls) or a solution of carotenoids in the drinking water daily ad libitum for 8 weeks. Males were sexually inexperienced young adults (all <1 year old), derived from 11 separate broods. All birds were judged to be healthy before and throughout the experiment in routine inspections carried out by experienced bird care and veterinary personnel. Birds were housed in their respective treatments in standard cages (60 × 45 cm and 50 cm high) at a density of either three individuals (in the case of nine brother-pairs) or two individuals per cage (two remaining brother-pairs). The laboratory environment was controlled at 20.5 ± 2.0°C, with full spectrum artificial light on a 16:8 h light:dark cycle (Bird Lamp; Arcadia, Croydon, U.K.). All males received a standard diet of seeds (foreign finch mixture; J. E. Haith, Cleethorpes, U.K.), grit and cuttlefish ad libitum. Lutein and zeaxanthin are the major dietary carotenoids in captive zebra finches on a seed diet (McGraw et al. 2002; Blount et al. 2003a). Control males received distilled water only, whereas carotenoid-supplemented males received 25 μg of carotenoids per ml of distilled water (Oro Glo liquid, 11 mg/ml lutein and zeaxanthin at a ratio of 20:1 (weight/weight), calorie-free; Kemin, Jalisco, Mexico). Since carotenoids are susceptible to oxidation on exposure to light, heat and oxygen, Oro Glo liquid was stored in a sealed container in the dark at 4°C, all drinks were prepared freshly once daily with prechilled water (4°C) and presented immediately to the birds in opaque dispensers (Blount et al. 2003b). This supplemental carotenoid dose was decided on because it falls towards the lower end of the wide range (12–200 μg carotenoids/ml water) known to increase blood levels of carotenoids and to increase immune responses after experimental challenge in captive studies of zebra finches (Blount et al. 2003b; McGraw & Ardia 2003; Alonso-Alvarez et al. 2004). It results in a mean carotenoid concentration of ca. 50 μg/ml of blood (Alonso-Alvarez et al. 2004). For comparison, reported blood carotenoid concentrations in captive zebra finches range from 13 to over 100 μg/ml of blood (McGraw & Ardia 2003; Alonso-Alvarez et al. 2004).

Brother-pairs were similar in morphology at the start of the experiment (paired t tests: body mass: $t_{10} = 0.93$, $P = 0.38$; tarsus length: $t_{10} = 0.01$, $P = 0.99$; wing length: $t_{10} = 0.71$, $P = 0.49$). We weighed birds with a digital balance (±0.01 g) and measured tarsus length with a sliding calliper and wing length (maximum chord) with a wing rule (±0.2 mm).

Tests of Flight Behaviour

We measured flight behaviour as described previously (Metcalfe & Ure 1995; Veasey et al. 1998), with some modifications. Trials were carried out immediately after 8 weeks of the diet manipulation in an indoor aviary (100 × 100 cm and 190 cm high). Prior to testing, birds were housed in the aviary for 48 h followed immediately by 24 h of rest in standard cages (as described above), then a further 24 h in the aviary to allow acclimation and to prime the birds physically to their individual optima (Metcalfe & Ure 1995; Veasey et al. 1998). The experimental diets were provided throughout this period: drinking water at perch height and seeds on the floor. Birds therefore had to visit the cage floor to forage, which encouraged flight activity. Flight trials were carried out by one of us (S.M.M.) blindly with respect to the treatment groups. Birds were captured and placed individually at the base of the aviary in an opaque, cylindrical chamber (14 cm diameter × 15 cm high) with a hinged, transparent Plexiglas lid. As a startle stimulus, the lid of the chamber was thrown open remotely with string making a loud clatter. The bird was then free to fly from the chamber to a perch 155 cm above the aviary floor, whereupon it was immediately recaptured by hand and the next trial was carried out. We carried out 10 trials for each bird. Some birds failed to emerge from the chamber within 3 s of the initial startle stimulus; in such cases the observer reached into the aviary and showed a hand at the lip of the chamber as a further startle stimulus. If the bird again failed to emerge within 3 s, it was removed from the chamber and released into the aviary, then immediately recaptured and replaced in the chamber for the next trial. Trials were recorded on to digital tape with a camcorder at 50 frames/s (Panasonic NV-DS27).

Data Retrieval and Analyses

Blindly with respect to the treatment group of birds, one of us (J.D.B.) viewed the video recordings and retrieved the following data: (1) Latency to emerge: the time elapsed between the chamber lid opening fully and the flying bird's bill becoming visible above the release chamber lip; (2) Flight time: the time elapsed between the flying bird's bill becoming visible above the release chamber lip and the bill reaching a mark on the aviary wall 70 cm above the floor (a height at the upper limit of the recorded field of view; (3) Relaunch: where birds punctuated their flight by perching momentarily on the release chamber lip before Relaunching to continue flying (scored on a 1/0 basis); (4) Hand stimulus: where a repeat (hand) stimulus was required to elicit flight (scored on a 1/0 basis); and (5) Failed flight: where the bird failed to take-off within 3 s of a hand stimulus (scored on a 1/0 basis).

Data for Latency to emerge and Flight time, respectively, were analysed by repeated-measures generalized linear mixed models (rmGLMM) in SAS release 8.02 (Proc Mixed; SAS Institute Inc., Cary, NC, U.S.A.), with diet (control or carotenoid) as a fixed categorical factor and trial (1−10) as a repeated-measures fixed categorical factor. We controlled for relatedness by including brother-pair as a random factor. Body mass is not affected by carotenoid supplementation in this species (Alonso-Alvarez et al. 2004; personal observation), but may nevertheless explain variation in flight performance (e.g. Witter & Cuthill 1993; Kullberg et al. 1996; Veasey et al. 1998). We therefore included body mass as a covariate in the analyses of Latency to emerge and Flight time. In the analysis of Flight time we also controlled for the effects of punctuated flight by including Relaunch as a fixed categorical factor. Variation in the density at which birds were housed during the period of dietary supplementation was balanced across treatments (see above), but nevertheless, for completeness, we tested whether such variation influenced escape flight responses. We found no statistically significant effects (all $P > 0.5$), and therefore housing density is not included as a factor in the analyses presented in this paper. All models were developed by backward elimination, starting with the highest order interaction term, and all possible interactions were tested. The factors brother-pair and Relaunch were always retained in final models. The covariance structure was defined as autoregressive (order 1) because this always gave the best model fit according to Akaike's Information Criterion, and degrees of freedom were calculated with Satterthwaite's correction (Littell et al. 1996). The significance of brother-pair (random factor) was assessed as the change in the −2 residual log likelihood upon factor exclusion, the resulting statistic having a chi-square distribution (Littell et al. 1996). Where appropriate, for post hoc analyses for the factor trial we used Tukey−Kramer tests.

Data for Latency to emerge and Flight time were non-normally distributed with a positive skew (Kolmogorov−Smirnov tests: Latency to emerge: $Z = 5.41$, $n = 203$, $P < 0.001$; Flight time: $Z = 6.09$, $N = 203$, $P < 0.001$) and heteroscedastic (F tests: Latency to emerge: $F_{97,106} = 4.64$, $P < 0.001$; Flight time: $F_{97,106} = 24.58$, $P < 0.0001$). After log transformation, data for Latency to emerge remained non-normally distributed ($Z = 4.16$, $N = 203$, $P < 0.001$) and heteroscedastic ($F_{97,106} = 3.29$, $P < 0.001$), while data for Flight time remained non-normally distributed ($Z = 4.71$, $N = 203$, $P < 0.001$) but were homoscedastic ($F_{97,106} = 1.27$, $P > 0.2$). Since there is no nonparametric test that can perform a repeated measures type ANOVA in instances where there are missing values (i.e. not all birds completed all 10 trials), we present the results of parametric rmGLMMs based on log values. Analysis of variance is robust to considerable departures from normality and homoscedasticity (Zar 1984) especially where results are statistically significant (Ito 1980). In addition, to verify the robustness of these analyses, both for Latency to emerge and Flight time, we used nonparametric Wilcoxon signed-ranks tests of the between-subjects factor, diet; the data were mean values for each treatment with trial (1−10) as the unit of replication.

There were too few positive values for Relaunch, Hand stimulus and Failed flight to permit repeated measures type analyses. Therefore, the total number of Relaunches divided by the total number of flights made is reported as the Relaunch ratio. Similarly, the total number of flights that followed Hand stimulus divided by the total number of flights made is reported as the Hand stimulus ratio. The total number of Failed flights divided by the total number of trials is reported as the Failed flight ratio. Values for each of these ratios were analysed with Wilcoxon signed-ranks tests with brother-pairs as matched subjects.

All P values are two tailed and alpha = 5%.

RESULTS

Carotenoid supplementation enhanced escape flight performance. Flight times were significantly shorter in carotenoid-supplemented males than in controls, and did not differ significantly over successive trials (Table 1, Fig. 1). Flight times also covaried with body mass similarly in both treatments, heavier birds taking longer (Table 1). The occurrence of Relaunching influenced Flight times (Table 1). However, this does not change the conclusion that carotenoid-supplemented birds had shorter Flight times. Relaunch ratios differed between treatments (Table 2), and the analysis of the effect of diet on Flight time statistically removed the effect of Relaunching by retaining this factor in the final model. In addition, compared with controls, carotenoid-supplemented birds less often required a repeat startle stimulus (Hand stimulus) to elicit escape flight (Table 2), and had a shorter Latency to emerge from the release chamber (Table 1, Fig. 1). The Latency to emerge was independent of body mass but varied according to trial number similarly in both diet treatments, increasing over successive trials (Table 1, Fig. 1). There was a significant effect of brother-pair in the analysis of Latency to emerge (Table 1), indicating that test responses of siblings were inherently similar. The Failed flight ratio tended to be lower in carotenoid-supplemented males than in controls, the difference being marginally statistically nonsignificant (Table 2).

Table 1. Variation in log($x + 1$)(Latency to emerge) and log(Flight time) arising from repeated measures mixed models with diet (control or carotenoid) and trial (1–10, repeated measure) as fixed categorical factors, and controlling for body mass (covariate) and relatedness of brother-pairs (random factor)

	Diet			Trial			Body mass			Relaunch		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Latency to emerge*	11.46	1,81.6	0.0011†	2.12	9,153	0.031‡	0.58	1,49.8	0.45	—	—	—
Flight time§	17.04	1,69.2	0.0001**	0.63	9,140	0.77	16.39	1,19.1	0.0007††	291.88	1,75.5	<0.0001

The analysis of Flight time also included Relaunch (0 or 1) as a fixed categorical factor to control for the occurrence of punctuated flights. All interactions were nonsignificant ($P > 0.11$).
*Effect of brother-pair: $\chi^2_1 = 12.0$, $P = 0.0006$.
†This conclusion was qualitatively the same in a nonparametric test (Wilcoxon test: $Z = 2.40$, $N = 10$, $P = 0.017$).
‡Post hoc Tukey–Kramer tests between all trials (1–10) failed to identify statistically significant differences (all $P > 0.08$). Therefore the mixed model was rerun comparing the first and last trial only as a repeated measures factor, which revealed that the Latency to emerge increased significantly over the course of the experiment (diet: $F_{1,16.3} = 6.35$, $P = 0.02$; trial: $F_{1,30} = 8.67$, $P = 0.006$; body mass: $F_{1,32.4} = 0.01$, $P = 0.91$; all interactions nonsignificant: $P > 0.43$).
§Effect of brother-pair: $\chi^2_1 = 1.5$, $P = 0.22$.
**This conclusion was qualitatively the same in a nonparametric test (Wilcoxon test: $Z = 2.60$, $N = 10$, $P = 0.009$).
††Parameter estimate: slope ± SE = 0.027 ± 0.007.

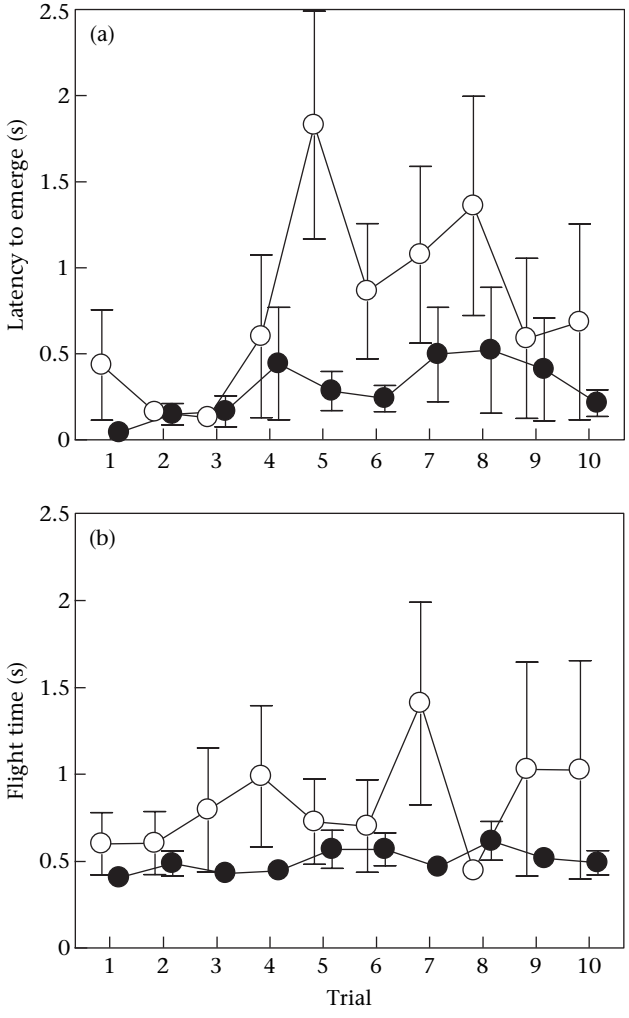

Figure 1. Flight take-off responses after a startle stimulus. (a) Latency to emerge; (b) Flight time. Values are means ± SE. ●: carotenoid-supplemented males; ○: control males. See Table 1 for results of statistical analyses.

DISCUSSION

We found that dietary carotenoid supplementation resulted in enhanced escape flight performance: carotenoid-supplemented males had significantly shorter Flight times than controls. In addition, compared with controls, carotenoid-supplemented birds less often required a repeat startle stimulus (Hand stimulus) to elicit escape flight, had a shorter Latency to emerge from the release chamber, and tended to fail to fly less often. Brothers were inherently similar in respect of the Latency to emerge, indicating an influence of genetic and/or maternal effects. However, despite this effect, the influence of the current environment (i.e. carotenoid availability) was clearly a strong determinant of escape flight responses.

A putative explanation for these results is that carotenoid supplementation enhanced the resistance of muscle tissue to oxidative damage, and hence reduced muscle susceptibility to fatigue during exercise. Reactive oxygen species produced during oxidative metabolism are the major cause of exercise-induced changes in muscle redox balance, which contributes to oxidative damage and muscle fatigue (reviewed in Powers et al. 2004). Because they had been in an aviary for 72 h immediately prior to tests of flight responses, having previously been housed in relatively small cages, individuals had to switch from making short hops and flights to far longer, near vertical flights to move between foraging and perching locations (see Methods). This opportunity for additional physical exercise may have increased muscle susceptibility to oxidative stress, controls being less able to defend against oxidative damage than carotenoid-supplemented birds. Rapid take-off at a steep angle is one of the most energetically demanding modes of flight in birds, requiring high burst power output from the pectoralis major muscles (Witter & Cuthill 1993). Repeated take-off can lead to exhaustion and an inability to fly (Askew & Marsh 2002). The fact that the Latency to emerge from the release chamber was higher in the last than in the first trial

Table 2. Variation in Relaunch ratios, Hand stimulus ratios and Failed flight ratios between control- and carotenoid-supplemented males arising from Wilcoxon signed-ranks tests (see Methods for details)

	Median (SD) range		Wilcoxon test		
	Control diet	Carotenoid diet	*Z*	*N*	*P*
Relaunch ratio	0.00 (0.32) 0–1	0.00 (0.27) 0–0.7	0.00	11	1.00
Hand stimulus ratio	0.30 (0.30) 0–0.86	0.00 (0.06) 0–0.13	2.38	11	0.02
Failed flight ratio	0.00 (0.27) 0–0.84	0.00 (0.13) 0–0.33	1.75	11	0.08

could reflect fatigue, but if so, why did carotenoid supplementation not mitigate this (i.e. the diet-by-trial interaction term was nonsignificant)? Temporal changes in the Latency to emerge may be explained at least in part by increasing levels of habituation to the startle stimulus, the kinetics of which would not be expected to be affected by carotenoid supplementation.

Earlier studies in vitro have shown that oxidative stress contributes to muscle fatigue, causing reduced exercise performance, which can be ameliorated by antioxidant activity (Barclay & Hansel 1991; Supinski et al. 1997; Powers et al. 2004). Support for the suggestion that dietary antioxidant supply can influence exercise performance in vivo has been equivocal. Supporting evidence comes from a few studies of rodents (Novelli et al. 1990, 1991; Devi et al. 2003) and humans (Simon-Schnass & Pabst 1988), weighed against a body of null evidence (reviewed in Powers et al. 2004). Many factors could account for these discrepancies, including inappropriate types and doses of supplemental antioxidants, and types and intensities of exercise performed (Powers et al. 2004). We are not aware of any study that has tested the effects of dietary antioxidant supply on exercise performance in birds. However, Møller et al. (2000) hypothesized that birds may be particularly susceptible to carotenoid limitation compared with other taxa because of a high requirement for and turnover of carotenoids. On average, birds typically have far higher blood concentrations of carotenoids than mammals do (Hill 1999). This is not simply because of differences in carotenoid levels in diets (Slifka et al. 1999), and therefore suggests that birds have a relatively greater need for carotenoids and hence have efficient carotenoid absorption and transport systems. The rate of carotenoid uptake and turnover in birds can be high, bodily levels increasing rapidly in response to dietary supplementation (e.g. Blount et al. 2003b; McGraw & Ardia 2003) and declining rapidly in response to immune sensitization (Allen 1997; Faivre et al. 2003; McGraw & Ardia 2003; Alonso-Alvarez et al. 2004). We therefore hypothesize that birds' flight muscles may be susceptible to antioxidant limitation, with negative consequences for exercise performance. To confirm that dietary carotenoid supplementation resulted in enhanced muscle resistance to oxidative damage would require that birds be killed for in vitro measurement of tissue oxidation products (protein carbonyls; malondialdehyde) in muscle (e.g. Supinski et al. 1997; Coombes et al. 2002). This deserves to be investigated, but was not an option in the present study because the birds were required for an experiment into the effects of carotenoid supply on life span.

Alternatively, carotenoid supplementation may have influenced motor performance or cognition. The vertebrate brain is rich in polyunsaturated fatty acids, and shows high levels of oxygen consumption and production of reactive oxygen species, making neurons readily susceptible to oxidative damage. Work on rodents has shown that administration of antioxidants can reduce oxidative damage in the brain and improve temporal and spatial memory (Carney et al. 1991; but see Sumien et al. 2004). This could be because antioxidant administration slows the rate of apoptosis of neurons, through either antioxidant protection or enhanced cellular signalling and transcriptional regulation (Ferri et al. 2003). Such mechanisms could have an important influence on the development of neurodegenerative diseases during the ageing process (Ferri et al. 2003). However, we are not aware of any studies showing that antioxidant administration can enhance motor performance or cognition in relatively young individuals. We must also consider the possibility that carotenoid supplementation, through effects on immune defences, could have resulted in birds being less debilitated by parasites or diseases and hence more capable of flight. Clearance of *Mycoplasma gallicepticum* bacterium after experimental inoculation has been shown to be faster in male house finches, *Carpodacus mexicanus*, with redder carotenoid-based plumage (Hill & Farmer 2005). Furthermore, dietary carotenoid supplementation has been shown to enhance immune defences specifically in zebra finches (Blount et al. 2003b; McGraw & Ardia 2003). It would be difficult, and in practical terms perhaps impossible, to assay for every possible parasite or disease that may be carried in a captive finch. An important point here is that the zebra finches used in this study were part of a large, well-established laboratory colony that has no history of serious pathogens, and were deemed to be healthy by experienced bird care and veterinary personnel before and during the experiment (see Methods). We therefore think it unlikely that carotenoid supplementation resulted in reduced debilitation by parasites or diseases.

We must also acknowledge the possibility that the supplemental carotenoid dose that we used (25 μg of carotenoids/ml of water) elevated blood carotenoid levels above naturally occurring levels. What constitutes a physiologically normal range for blood carotenoids in zebra finches is uncertain because as yet there are no data for wild birds. In a captive study, McGraw & Ardia (2003) reported ranges of 13–75 μg of carotenoids/ml of plasma in nonsupplemented birds and 13–82 μg/ml in carotenoid-supplemented birds. These

ranges are a product of both the basal diet and the supplement, and of course these two factors vary among captive populations and studies. Far higher supplemental carotenoid doses have been used in other studies of passerines (1000 μg of carotenoids/ml of water in American goldfinches, *Carduelis tristis*; Navara & Hill 2003), including zebra finches (200 μg of carotenoids/ml of water; Alonso-Alvarez et al. 2004). Importantly, blood carotenoid levels continue to rise in zebra finches in response to increasing dietary carotenoid intake until they reach at least 100 μg of carotenoids/ml of drinking water, and at this dose blood carotenoid concentrations range from ca. 30 to 130 μg/ml (Alonso-Alvarez et al. 2004). Therefore, despite a 10-times higher carotenoid dose, the variance in blood carotenoid levels among individuals is similar to that found by McGraw & Ardia (2003). Ultimately, to determine the physiologically normal range for blood carotenoids in zebra finches requires study of wild individuals.

Clearly there are several potential explanations for how carotenoids may have influenced escape flight responses; indeed a combination of antioxidant and nonantioxidant mechanisms could be responsible. The range of biological activities of carotenoids is still not fully understood (Yeum & Russell 2002), and in particular the contribution of carotenoids to intercellular signalling, inhibition of regulatory enzymes and gene expression needs further study (Stahl et al. 2002). It is also important to raise the potential caveat that behaviour in captive zebra finches may not be a good predictor of behaviour in wild zebra finches. Because of their history of domestication, and relatively limited scope for exercise in the laboratory, captive zebra finches may have reduced flight capacity compared with wild conspecifics. It is therefore unclear whether we should expect differences between wild and captive birds in the degree to which flight performance may be constrained by carotenoid availability. The main conclusion that may be drawn from this study is that one measure of physiological performance at the behavioural level, escape flight responses in captive finches, can be constrained by carotenoid availability. This raises the possibility that carotenoid-dependent sexual signals in males may reveal their capacity for exercise to prospective mates. Flight take-off performance is thought to be a key determinant of the ability to evade capture by predators and to forage, and should therefore be a major target for natural selection (Witter & Cuthill 1993; Metcalfe & Ure 1995; Kullberg et al. 1996; Veasey et al. 1998). It may also predict the capacity to provide parental care, because this depends in part on parental survival and foraging ability. Flight capacity may be particularly important in zebra finches, a species that breeds opportunistically soon after heavy rainfall when food supplies become favourable, and therefore must increase its work rate to build nests, forage and care for eggs and chicks at short notice. Indeed, predation and starvation are major selection agents in this short-lived species (Zann 1996). Correlational studies in other species have shown that individuals with greater carotenoid pigmentation have superior foraging success (for carotenoids; Hill et al. 2002) and work harder at provisioning mates (house finches: Hill 1991) and nestlings (northern cardinals, *Cardinalis cardinalis*: Linville et al. 1998). These relations remain to be studied in zebra finches. It would therefore now be interesting to test experimentally whether variation in carotenoid supply influences the provision of paternal care in the field.

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