inspection of type material from the Eocene Río Pichileufú flora (*58*) confirms the reported taxonomic similarity to the LH flora (*26*), and our preliminary field data from Río Pichileufú suggest a diversity comparable to LH. Formal taxonomic knowledge of the LH flora is not yet sufficient to allow rigorous analysis of familial and generic diversity (*34*, *36*). All 1215 voucher specimens for this study are housed at MEF, including exemplar specimens of each species. Fieldwork reported here took place in November 1999. We do not attempt to integrate our data with previous collections in the United States and Argentina, which would be inappropriate for our stratigraphic methodology.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/300/5616/122/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 to S7
References and Notes

14 November 2002; accepted 11 February 2003

Carotenoid Modulation of Immune Function and Sexual Attractiveness in Zebra Finches

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One hypothesis for why females in many animal species frequently prefer to mate with the most elaborately ornamented males predicts that availability of carotenoid pigments is a potentially limiting factor for both ornament expression and immune function. An implicit assumption of this hypothesis is that males that can afford to produce more elaborate carotenoid-dependent displays must be healthier individuals with superior immunocompetence. However, whether variation in circulating carotenoid levels causes variation in both immune function and sexual attractiveness has not been determined in any species. In this study, we show that manipulation of dietary carotenoid supply invokes parallel changes in cell-mediated immune function and sexual attractiveness in male zebra finches (*Taeniopygia guttata*).

Females in many animal species frequently prefer to mate with the most elaborately ornamented males (*1*), but how such displays reveal a male's worth is a contentious issue (*2*–*5*). For a signal to honestly reveal an individual's quality, it must be costly to produce (*6*, *7*). The expression of many ornamental traits depends on carotenoids, red and yellow pigments that animals cannot synthesize de novo and ultimately must obtain through their diet (*2*–*5*). Carotenoids are antioxidants and immunostimulants (*8*, *9*), and it has recently been hypothesized that a trade-off exists in carotenoid allocation between maintaining health and ornamentation: Males in better condition should require fewer carotenoids for immune function and could therefore use more of this resource to enhance ornamental display, thereby advertising their superior health (*10*, *11*). However, whether variation in

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circulating carotenoid levels directly causes variation in both immune function and sexual attractiveness has not been determined in any species (*2–4*).

We used zebra finches to test the predictions (*10*, *11*) that immune function is limited by carotenoid availability in a species with carotenoid-dependent ornamentation and that females prefer the most carotenoid-rich males. In a matched-pairs experimental design, we randomly allocated 10 pairs of adult, full-sibling zebra finch brothers to receive either distilled drinking water (controls) or carotenoids (lutein and zeaxanthin) in the drinking water daily ad libitum over a period of 8 weeks (*12*). These are among the major specific plasma carotenoids found in captive zebra finches on a seed diet (*13*). Males in both treatments received ad libitum white millet, which contains lutein and zeaxanthin (*14*), and all other experimental conditions were standardized (*12*). Bill coloration is a carotenoid-based (*13*), condition-dependent secondary sexual trait in zebra finches (*15–17*). To assess the kinetics of equilibration of the birds to the experimental diets at the start of the experiment and again after 4 and 8 weeks, we ranked bill color under standardized conditions by reference to a chart of color chips ranging from 1 (light orange) to 9 (dark red) (*12*). Brother-dyads did not differ significantly in bill color at the start of the experiment (Wilcoxon's matched-pairs test, $Z = 0.91$, $P = 0.36$). Although carotenoid supplementation resulted in significantly increased bill color rank, mainly over the first 4 weeks, there was no change in bill color over the course of the experiment in controls (Fig. 1). Consequently, carotenoid-supplemented males had developed significantly redder bills compared with controls by week 4 ($Z = 2.09$, $P = 0.036$), and this effect was sustained for the duration of the experiment (week 8: $Z = 2.71$, $P = 0.007$).

After 6 weeks of the experimental diets, we tested whether males of the two treatments differed in their attractiveness to females by measuring the relative proportion of time that nonexperimental females spent perched next to the males (*12*). Females preferred carotenoid-supplemented males to their control brothers (Fig. 1). Which specific aspects of male phenotype were responsible for influencing the patterns of female mate choice is not known. It has been shown that female zebra finches prefer males with redder bills (*15*, *16*) but also those with higher display rates (*18–20*), a trait hypothesized to be antioxidant-dependent (*11*). Twelve to 14 days later, during week 8 of the experimental diets, we collected a blood sample for analysis of plasma carotenoid levels by high-performance liquid chromatography (*12*). Then, 2 days later, we measured in vivo cell-mediated immune responses with the use of phytohemagglutinin (PHA) injected into the wing web (*12*). PHA is a lectin that induces a nonspecific mitogenic response of T lymphocytes, resulting in perivascular accumulation of various leukocytes and thickening of the skin (*21*) that can be measured as a swelling when injected intradermally (*12*). Carotenoid-supplemented males had significantly higher levels of circulating carotenoids, and they produced significantly larger PHA responses than controls (Fig. 2). Cell-mediated immunity is believed to have important life-history consequences; intraspecific studies of birds have shown that individuals with larger PHA responses have greater survival (*17*, *22*).

These results from male zebra finches demonstrate that variation in body levels of carotenoids causes parallel variation in cell-mediated immune function and sexual attractiveness, providing direct support for the hypothesis (*10*, *11*) that females choosing to mate with the most carotenoid-rich males stand to acquire a more immunocompetent mate. The direct benefits of such mating decisions to females could include a lower risk of becoming infected with parasites and diseases and a greater likelihood that the chosen male can provide adequate parental care if required (*10*). Because immune and antioxidant defenses partly have a genetic basis, there is also the intriguing possibility that a choosy female may obtain viability genes for her offspring that confer enhanced efficiency in resistance to parasites, diseases (*23*), and oxidative stress (*11*). We found no evidence to suggest that brothers had inherently similar plasma carotenoid levels (table S1), suggesting that variation in carotenoid uptake from the diet is not strongly influenced by genetic (or maternal) effects. However, genes could plausibly shape other stages of the carotenoid acquisition and utilization process, such as an individual's foraging or immune efficiency.

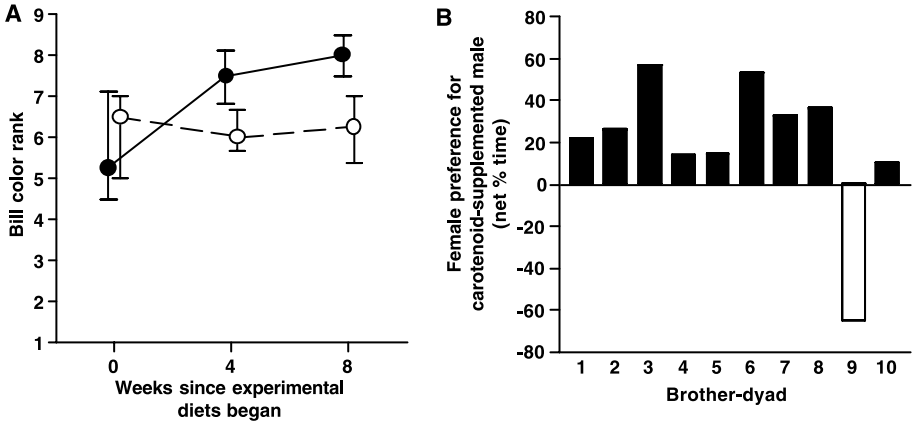

Fig. 1. Bill coloration and attractiveness of males in relation to experimental diet (control males, open symbols; carotenoid-supplemented males, closed symbols). (**A**) Bill color rank (median, first and third quartiles), which increased in carotenoid-supplemented males, mainly over the first 4 weeks [Friedman's analysis of variance (ANOVA) by ranks, $\chi^2 = 15.39$, df = 2, $P < 0.0001$; posthoc comparisons, 0 versus 4 weeks, $Z = 2.81$, $P = 0.005$; 4 versus 8 weeks, $Z = 1.87$, $P = 0.06$], but did not change in controls ($\chi^2 = 0.06$, df = 2, $P = 0.97$). (**B**) Female mate choice (percentage of time perched next to carotenoid-supplemented minus control male). Nine out of 10 females preferred carotenoid-supplemented males (sign test, $P = 0.021$).

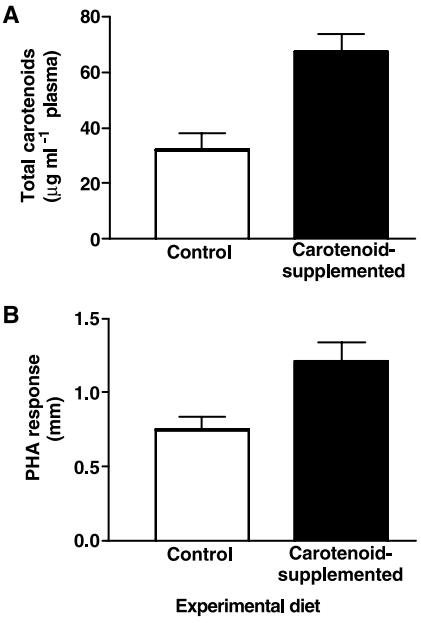

Fig. 2. Effects of experimental diet on (**A**) plasma total carotenoid concentrations and (**B**) PHA responses [values are means (±SEM)]. Carotenoid supplementation resulted in significantly increased plasma carotenoid concentrations ($F = 11.11$; df = 1, 9; $P = 0.009$) and also PHA responses ($F = 7.86$; df = 1, 9; $P = 0.021$). Analyses are randomized-complete-blocks ANOVAs for paired comparisons (table S1).

Various alternative hypotheses have been proposed to explain the signal content of carotenoid-dependent ornaments (*2*–*5*). The relative importance of carotenoid acquisition per se, as influenced by foraging efficiency (*24*, *25*), parasite effects on gut absorption (*26*), energetic constraints (*27*), and carotenoid utilization for immune function, in determining the expression of sexual ornaments remains to be seen. However, our results show that immune function can be limited by carotenoid availability in a species with carotenoid-dependent ornamentation and suggest that immunocompetence is one trait that is revealed by the expression of such signals.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/300/5616/125/DC1
Materials and Methods
Table S1

7 January 2003; accepted 6 March 2003

Visualizing tmRNA Entry into a Stalled Ribosome

Mikel Valle,[1]* Reynald Gillet,[2]* Sukhjit Kaur,[1] Anke Henne,[3] V. Ramakrishnan,[2]† Joachim Frank[1,4]†

Bacterial ribosomes stalled on defective messenger RNAs (mRNAs) are rescued by tmRNA, an ~300-nucleotide-long molecule that functions as both transfer RNA (tRNA) and mRNA. Translation then switches from the defective message to a short open reading frame on tmRNA that tags the defective nascent peptide chain for degradation. However, the mechanism by which tmRNA can enter and move through the ribosome is unknown. We present a cryo–electron microscopy study at ~13 to 15 angstroms of the entry of tmRNA into the ribosome. The structure reveals how tmRNA could move through the ribosome despite its complicated topology and also suggests roles for proteins S1 and SmpB in the function of tmRNA.

During the normal course of protein synthesis, a problem occurs if the ribosome reaches the 3′ end of a defective or degraded mRNA before it encounters a stop codon. This situation has two possible consequences: the ribosome can stall, and the incomplete polypeptide made as a result may be toxic to the cell. In bacteria, both these problems are solved simultaneously by the intervention of an RNA molecule called 10Sa RNA, SsrA, or most commonly, tmRNA, because it incorporates within a single molecule the functions of both tRNA and mRNA (*1*–*3*). The tmRNA molecule is ~260 to 430 nucleotides long, depending on bacterial species, and contains both a tRNA-like domain (TLD) that can be charged with alanine at its 3′ CCA end and an internal stretch of RNA that contains a short open reading frame (ORF). The molecule first

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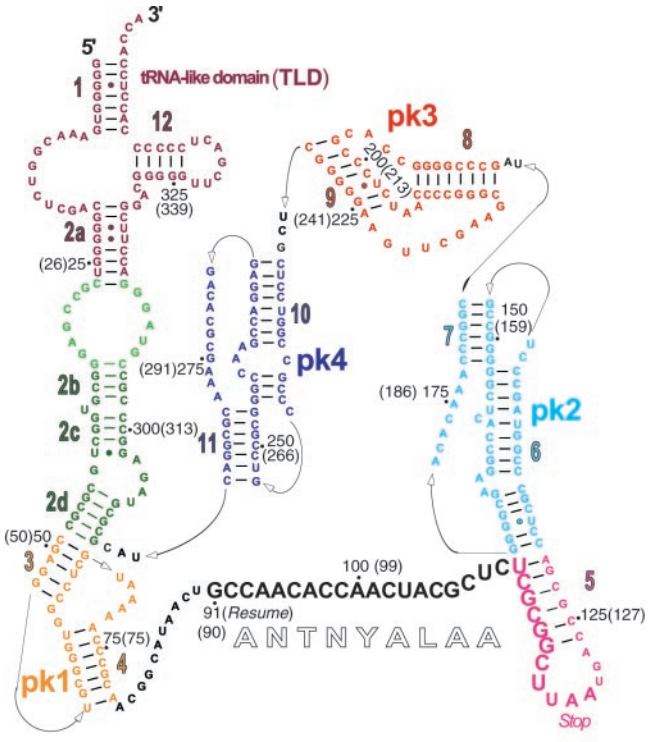

tmRNA (Thermus thermophilus)

Fig. 1. Secondary structure diagram of tmRNA. Base pairs are linked by lines, whereas GU pairs are represented by dots. The TLD, the four pseudoknots (PK1 to PK4), and helices (numbered from 1 to 12) are shown in the colors that are used to represent these modules in subsequent figures. The nucleotides within the ORF are shown in a larger font. *T. thermophilus* sequence numbers are shown, with the corresponding *E. coli* numbering in parentheses. The figure is adapted from the diagram for *E. coli* on the tmRDB Web site (*29*, *41*).