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CAROTENOIDS IN THE SEMINAL FLUID OF WILD BIRDS: INTERSPECIFIC VARIATION IN FAIRY-WRENS

MELISSAH ROWE[1,3] AND KEVIN J. MCGRAW[2]

[1]*Department of Ecology and Evolution, University of Chicago, 1101 East 57th Street, Chicago, IL 60637*
[2]*School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501*

Abstract. Male secondary sexual characters can provide females with information regarding the fertilizing capacity of a male's sperm. In some fishes and birds, intense nuptial coloration is correlated with male fertilizing capacity, but no mechanistic link between coloration and sperm quality has been established. One plausible mechanism is that carotenoid pigments, which color skin and feathers in many animals, are present in seminal fluid and serve as antioxidant protectors of sperm. We used high-performance liquid chromatography (HPLC) to analyze sperm samples from four species of Australian fairy-wren (*Malurus*) and detected low concentrations (<1 μg ml^{-1}) of carotenoids in some samples. Xanthophyll carotenoids (including lutein and zeaxanthin), which are typically dietary in origin, were present in the seminal fluid of Superb (*M. cyaneus*) and Splendid (*M. splendens*) Fairy-Wrens. In contrast, red ketocarotenoids (including astaxanthin and canthaxanthin), which are likely metabolically derived from dietary precursors, were present in the seminal fluid of Red-backed Fairy-Wrens (*M. melanocephalus*). This work is the first to report carotenoids in avian seminal fluid and suggests that, although carotenoids are at low levels and thus may have limited antioxidant activity, there may be biological variability in avian semen carotenoids on which selection could act.

Key words: antioxidants, carotenoid pigments, Malurus, *oxidative stress, sexual competence, sperm, sperm competition.*

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Resumen. Los caracteres sexuales secundarios de los machos pueden brindar a las hembras información sobre la capacidad de fertilización del esperma de un macho. En algunos peces y aves, la coloración nupcial intensa se correlaciona con la capacidad de fertilización del macho, pero no se ha establecido un mecanismo que vincule la coloración con la calidad del esperma. Un mecanismo posible es que los pigmentos carotenoides, que colorean la piel y las plumas en muchos animales, estén presentes en el fluido seminal y sirvan como antioxidantes protectores del esperma. Empleamos cromatografía líquida de alto rendimiento para analizar muestras de esperma provenientes de cuatro especies de *Malurus* y detectamos bajas concentraciones (<1 μg ml^{-1}) de carotenoides en algunas muestras. Los carotenoides xantófilos (incluyendo luteína y zeaxantina), que son típicamente de origen dietario, estuvieron presentes en el fluido seminal de *M. cyaneus* y *M. splendens*. En contraste, los quetocaroteniodes rojos (incluyendo astaxantina y cantaxantina), que son probablemente derivados metabólicos de precursores dietarios, estuvieron presentes en el fluido seminal de *M. melanocephalus*. Este trabajo es el primero en describir la presencia de carotenoides en el fluido seminal de aves y sugiere que, aunque los carotenoides estén en bajos niveles y por ende puedan tener actividad antioxidante limitada, puede haber variabilidad biológica en los carotenoides del semen de las aves sobre la cual podría actuar la selección.

INTRODUCTION

Carotenoids are naturally occurring fat-soluble pigments that color the skin and feathers of many vertebrates, and these colorful traits are often the target of sexual or social selection (Hill 1999, Karubian 2002, Blount et al. 2003). Carotenoids also appear to be important antioxidants and immunostimulants, quenching reactive oxygen species (free radicals), minimizing oxidative stress, and stimulating cell-mediated immune responses (Miki 1991, Bendich 1993, Blount et al. 2003, McGraw and Ardia 2003, but see Constatini and Møller 2008). Because animals cannot synthesize carotenoids de novo, but must acquire them from dietary sources, carotenoid acquisition is a potentially limiting factor for the expression of carotenoid-based color ornaments (Hill et al. 2002). Furthermore, because carotenoids may be adaptively allocated to multiple functions, such as coloration and immune defense, it is argued that only high-quality males can acquire sufficient carotenoid resources for maximizing both health and coloration (Lozano 1994, Olson and Owens 1998, von Schantz et al. 1999, but see Fitze et al. 2007, Isaksson and Andersson 2008). Ultimately, as males may vary in their ability to acquire, utilize, or

Manuscript received 3 April 2008; accepted 16 September 2008.
[3]Present address: School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501.
E-mail: melissah.rowe@asu.edu

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allocate carotenoid resources, carotenoid-based coloration and ornaments are often considered to be honest, condition-dependent indicators of male quality (Hill 1991, Blount et al. 2003, Pike, Blount, Bjerkeng et al. 2007, Pike, Blount, Lindström, and Metcalfe 2007).

Male ornamentation may also reveal characteristics of fertilizing capacity, namely sperm quantity or quality. The idea that a male trait may provide information about a male's sexual competence was initially viewed as a link between male vigor during courtship and high sperm supplies (Trivers 1972). More recently, the phenotype-linked fertility hypothesis proposed that male fertility and phenotype covary, and that fertility can be signaled to females via the expression of sexual ornaments (Sheldon 1994). These hypotheses suggest that female preference for male phenotypic traits is a result of direct selection for fertility benefits to females, or indirect selection for superior fertilizing capacity in sons (Trivers 1972, Sheldon 1994, Pizzari et al. 2004).

Blount et al. (2001) suggested that antioxidants, including carotenoids, might provide a mechanistic link between male ornamentation and sperm quality if both sperm and the ornament demand antioxidant pigments. In vertebrates, exposure of sperm to reactive oxygen species results in sperm damage, including modification of the cytoskeleton and damage to sperm axoneme, which translates into reduced sperm motility (de Lamirande and Gagnon 1992). Furthermore, the production of free radicals decreases sperm motility and inhibits sperm-oocyte fusion, resulting in a loss of fertility (Aitken et al. 1989, Iwasaki and Gagnon 1992). Avian sperm appear to be particularly susceptible to oxidation-induced damage (Surai et al. 1998, 2001), and antioxidant protection appears to be vital in maintaining the fertilizing capacity of avian sperm (Fujihara and Howarth 1978, Surai et al. 2001). If present, seminal-fluid carotenoids could potentially function as antioxidants to prevent sperm damage and maintain the fertilizing capacity of a male's sperm. Moreover, male ornamentation and sperm quality may be linked if there is a trade-off in terms of allocation of carotenoid resources between the two biological functions (i.e., ornament expression and sperm antioxidant defense)—similar to the tenet proposed for carotenoid allocation to the immune system (Lozano 1994)—and if males vary in their ability to acquire carotenoid resources. This suggests that high-quality individuals able to accumulate larger carotenoid reserves can allocate greater carotenoid resources to sperm protection as well as produce more elaborate carotenoid-dependent coloration and thus advertise their superior sexual competence.

The potential for carotenoid-based colors to signal male fertility has recently received support in some fishes and birds. In Mallards (*Anas platyrhynchos*), the carotenoid-based bill color of males is correlated with plasma carotenoid concentration, sperm velocity, and immune response (Peters et al. 2004). In guppies (*Poecilia reticulata*), carotenoid-based nuptial coloration is associated with several measures of sexual competence, including sperm quantity, sperm motility, and sperm length (Pitcher et al. 2007). These studies, however, do not demonstrate a mechanistic link between male coloration and sperm quality, nor have they documented the presence or action of carotenoid pigments in seminal fluid. In fact, studies examining the presence of carotenoids in the semen of animals are limited to a few studies of humans (*Homo sapiens*), fish, and invertebrates (Czeczuga 1975, Palan and Naz 1996, Heller et al. 2000, Goyal et al. 2007). To our knowledge, no studies to date have reported the presence of carotenoids in the seminal fluid of any bird species, which is a key assumption to validate before further studies examining the association between male carotenoid ornamentation and sperm quality in birds are undertaken.

In this study, we used high-performance liquid chromatography (HPLC) to examine the carotenoid content of sperm from four species of Australian fairy-wren (*Malurus*): the Superb (*M. cyaneus*), Splendid (*M. splendens*), Variegated (*M. lamberti*), and Red-backed (*M. melanocephalus*) Fairy-Wrens. Fairy-wrens are small (c. 6–10 g), cooperatively breeding, insectivorous passerines distributed throughout Australia and New Guinea (Rowley and Russell 1997). Within the Australian fairy-wrens, three distinct phylogenetic clades are recognized: the blue *cyaneus* group (including the Superb and Splendid Fairy-Wrens), the chestnut-shouldered *lamberti* group (including the Variegated Fairy-Wren), and the bicolored *leucopterus* group (including the Red-backed Fairy-Wren; Christidis and Schodde 1997). Many species of fairy-wren exhibit strong sexual dimorphism in plumage coloration, with males exhibiting bright nuptial coloration during the breeding season. The mechanism of plumage coloration varies across species: Superb, Splendid, and Variegated Fairy-Wrens display structural- and melanin-based plumage coloration, whereas Red-backed Fairy-Wrens display melanin-based, black plumage and what is presumed to be carotenoid-based, red plumage (Rowley and Russell 1997). Fairy-wrens are an ideal group for studies of sperm quality and sexual selection because some species, notably the Superb, Splendid, and Red-backed Fairy-Wrens, exhibit some of the highest levels of extra-pair paternity recorded to date (Dunn and Cockburn 1999, Karubian 2002, Webster et al. 2004). Such high levels of extra-pair paternity imply that the majority of females within a population copulate with two or more males during a single reproductive episode and, as a result, the sperm from multiple males compete for fertilization of a female's ova (Birkhead and Møller 1992). In birds, the outcome of sperm competition is determined by the timing of copulation, the number of sperm transferred during copulation, and the fertilizing capacity of sperm (Birkhead and Pizzari 2002). Therefore, mechanisms that influence male fertilizing capacity, such as the antioxidant protection of sperm by carotenoids, would likely be important in species such as fairy-wrens that experience intense sperm competition.

METHODS

FIELD METHODS AND SAMPLE COLLECTION

Seminal fluid samples were collected from Superb Fairy-Wrens in October 2005, from Splendid Fairy-Wrens in November 2005 and 2006, from Variegated Fairy-Wrens in November 2006, and from Red-backed Fairy-Wrens in December 2006. Superb Fairy-Wrens were studied at Murray River National Park, South Australia (140°32′E, 34°20′S), and both Splendid and Variegated Fairy-Wrens were studied at Brookfield Conservation Park, South Australia (139°29′E, 34°20′S). Red-backed Fairy-Wrens were studied at two sites, Kalinvale Farm and Moomin Reservoir, near Herberton, Queensland (145°23′E, 17°23′S). All individuals were trapped by herding focal individuals into mist nets. Semen was collected using standard cloacal massage techniques (Quinn and Burrows 1936, Tuttle et al. 1996). Exuded semen was collected in calibrated microcapillary tubes and immediately wrapped tightly in foil to prevent light exposure. In 2005, semen samples were stored in 100% ethanol and transported to the United States for analysis. In 2006, we had access to liquid nitrogen; consequently semen samples were stored in liquid nitrogen, transported back to the United States on dry ice, and stored in a –80°C freezer until later analysis.

For Red-backed Fairy-wrens, we also collected 8 plasma and 11 feather samples from primary breeding males exhibiting red and black nuptial plumage so that we could compare carotenoid profiles among tissues. A small blood sample (c. 20–80 µl) was collected from the wing or tarsus vein in heparinized microcapillary tubes. The tubes were centrifuged using a capillary rotor for 5 min at 2000 rpm, after which time the plasma was separated from the packed cells and stored in liquid nitrogen, transported to the United States on dry ice, and then stored in a –20°C freezer. For feather samples, we removed six to eight red feathers from the backs of males and stored these feathers in dry, sealed glassine envelopes held in the dark at room temperature until later analysis. Feather and plasma samples were not analyzed for Superb, Splendid, or Variegated Fairy-Wrens because these species do not display carotenoid-based plumage and because plasma samples were unavailable.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

To ensure sufficient sample size for analysis, we pooled semen from two or more males to obtain pooled samples with a total volume >10 µl. We analyzed 6 samples from the Superb Fairy-Wren, 14 samples from the Splendid Fairy-Wren, 1 sample from the Variegated Fairy-Wren, and 7 samples from the Red-backed Fairy-Wren. Chemical extraction methods for carotenoids in seminal fluid followed those described by McGraw et al. (2006) for avian plasma. We added 100 µl ethanol to 10 µl semen and vortexed the solution for 5 sec. We then added 100 µl tert-butyl methyl ether to the tube and vortexed again for 5 sec. We centrifuged the solution at 12 000 rpm for 1 min and transferred the supernatant to a fresh tube. The solvent was evaporated to dryness and the pigment residue resuspended in 200 µl mobile phase (42:42:16, methanol:acetonitrile:dichloromethane, volume/volume/volume) prior to HPLC analysis. Methods for extraction of plumage and plasma pigments also follow those by McGraw et al. (2006).

Following McGraw et al. (2006), we used a Waters Corporation (Milford, Massachusetts) Alliance HPLC system equipped with a Carotenoid C-30 (Waters Corporation) column to determine types and amounts of carotenoids present. The gradient method described by McGraw et al. (2006) was modified slightly to cut down on run times: initial isocratic conditions were maintained until minute 11, at which point we began running the linear gradient until minute 21. Final gradient conditions were then held until minute 25 and then returned to initial isocratic conditions until minute 29.5. Carotenoids were identified by comparison to authentic reference carotenoids, using both retention time and light-absorption maxima as diagnostic characteristics. Data were collected from 250–600 nm using a 2996 photodiode array detector (Waters Corporation). The detection limit for carotenoid pigments was 0.001 µg ml^{-1}.

STATISTICAL ANALYSES

Because we had only a single seminal fluid sample from Variegated Fairy-Wrens, we excluded this species from statistical analyses. Data were not normally distributed (Shapiro-Wilk normality test) and transformations failed to normalize distributions, so we used nonparametric statistical tests throughout the analyses. We used the Kruskal-Wallis rank sum test to determine if species differed in total concentration of carotenoids in seminal fluid samples, plus a post-hoc Behrens-Fisher test to examine all pairwise comparisons among the three species (Munzel and Hothorn 2001). To determine if the concentrations of xanthophyll carotenoids—lutein and zeaxanthin—differed between Splendid and Superb Fairy-Wrens, we performed a Wilcoxon rank sum test for each carotenoid type. All statistical analyses were conducted using the R (2.4.1) software package (R Development Core Team 2006). An alpha level of 0.05 was used for all tests. Values reported are means ± SE.

RESULTS

Upon collection in the field and prior to carotenoid extraction in the lab, fairy wren seminal fluid appeared white- or cream-colored to the naked eye. Carotenoids were detected in 83% of Superb Fairy-Wren samples, 36% of Splendid Fairy-Wren semen samples, and 71% of Red-backed Fairy-Wren samples. We did not detect carotenoids in the single sample of seminal fluid from the Variegated Fairy-Wren. In the seminal fluid of Superb and Splendid Fairy-Wrens, two xanthophyll carotenoids were present: lutein and zeaxanthin (Table 1, Appendix). We identified only red ketocarotenoids—astaxanthin, canthaxanthin, and adonirubin—in the seminal fluid of Red-backed Fairy-Wrens

TABLE 1. The concentration of carotenoids in the seminal fluid of four species of Australian fairy-wren (genus *Malurus*). Seminal fluid samples were collected from Superb Fairy-Wrens (*Malurus cyaneus*) in 2005, from Splendid Fairy-Wrens (*M. splendens*) in 2005 and 2006, and from Variegated Fairy-Wrens (*M. lamberti*) and Red-backed Fairy-Wrens (*M. melanocephalus*) in 2006. Samples were analyzed using high-performance liquid chromatography (HPLC). Values (means ± SE) are in μg ml^{-1}.

Species	Lutein	Zeaxanthin	Adonirubin	Canthaxanthin	Astaxanthin	Total carotenoids
Superb ($n = 6$)	0.37 ± 0.1	0.10 ± 0.02	0	0	0	0.47 ± 0.12
Splendid ($n = 14$)	0.07 ± 0.04	0.02 ± 0.02	0	0	0	0.08 ± 0.06
Variegated ($n = 1$)	0	0	0	0	0	0
Red-backed ($n = 7$)	0	0	0.07 ± 0.03	0.10 ± 0.04	0.06 ± 0.03	0.24 ± 0.09

(Table 1, Appendix). It is possible that lutein and zeaxanthin are also present in seminal fluid, as they are present in circulation, but occur at concentrations below our detection limit.

Total carotenoid concentration in seminal fluid differed significantly among Superb, Splendid, and Red-backed Fairy-Wrens ($\chi^2_2 = 7.6$, $P = 0.02$; Table 1). Levels were lower in Splendid Fairy-Wrens compared to Superb Fairy-Wrens ($P = 0.05$) but not to Red-backed Fairy-Wrens ($P = 0.25$). Concentrations did not differ significantly between Red-backed and Superb Fairy-Wrens ($P = 0.31$). Semen from Superb Fairy-Wrens had significantly higher levels of both lutein ($W = 13.5$, P = 0.01) and zeaxanthin ($W = 12.5$, P = 0.003) than that of Splendid Fairy-Wrens.

Carotenoids in plasma and feather samples from male Red-backed Fairy-Wrens were more concentrated and diverse than those in their semen (Table 2). We identified six carotenoids in plasma, including two xanthophyll carotenoids—lutein and zeaxanthin—and four red ketocarotenoids: alpha-doradexanthin, astaxanthin, canthaxanthin, and adonirubin (Table 2, Appendix). Concentration of seminal fluid carotenoids represented only 1%–2% of levels found in plasma. In feathers, we identified two yellow carotenoids (canary xanthophylls A and B) as well as the four ketocarotenoids found in plasma (Table 2, Appendix), confirming that coloration of the red feathers in this species is indeed carotenoid-based.

TABLE 2. The concentration of carotenoids in plasma and feather samples from the Red-backed Fairy-Wren (*Malurus melanocephalus*). Red-backed Fairy-Wrens were studied near Herberton, Queensland, Australia during December 2006. Plasma and feather samples were analyzed using high-performance liquid chromatography (HPLC). Values are means ± SE. Plasma values are in μg ml^{-1}; feather values are in μg g^{-1}.

Pigment	Plasma ($n = 8$)	Feathers ($n = 11$)
Canary xanthophylls A	0	39.24 ± 10.80
Canary xanthophylls B	0	41.27 ± 10.85
Alpha-doradexanthin	3.61 ± 1.24	130.02 ± 30.96
Astaxanthin	3.60 ± 0.68	257.65 ± 43.17
Canthaxanthin	5.16 ± 1.52	414.33 ± 65.72
Adonirubin	4.18 ± 0.77	367.72 ± 64.33
Zeaxanthin	0.52 ± 0.22	0
Lutein	1.21 ± 0.37	0
Total carotenoids	18.27 ± 4.36	1227.91 ± 197.48

DISCUSSION

To our knowledge, this study provides the first qualitative and quantitative report of carotenoid pigments in the seminal fluid of birds. Analyses of carotenoid pigments in avian tissues have previously been conducted on the brain, liver, adipose, plasma, immune tissues, eyes, gonads, and integument (McGraw 2006). However, it has never been documented whether carotenoids are present in the seminal fluid of any avian species, despite the exciting suggestion by Blount et al. (2001) that allocating carotenoids to seminal fluid may protect sperm from free-radical attack and may link male fertility and sperm quality to ornamental display.

The concentrations of the carotenoid pigments we detected, however, were very low, and in some cases carotenoids were not detected. A similar dilute amount of carotenoids (xanthophylls) are also present in the seminal fluid of domestic chickens (*Gallus gallus domesticus*; KJM, pers. obs.). The overall average total concentration of carotenoids in the seminal fluid of fairy-wrens was very low (mean = 0.21 μg ml^{-1}, range: 0–0.85 μg ml^{-1}) when compared to other tissues from wild birds (Surai 2002, McGraw 2006). Similarly, the carotenoid concentrations in semen were considerably lower than those in the plasma and feathers of Red-backed Fairy-Wrens. Plasma carotenoid levels less than 1 μg ml^{-1} are rare in wild birds (Tella et al. 1998, 2004), although captive birds maintained on carotenoid-deficient diets can exhibit such low levels (McGraw 2005).

The low concentration of carotenoid pigments in the seminal fluid of fairy-wrens suggests that carotenoids may have a limited role in antioxidant defense of sperm. Typically, carotenoids are thought to be less-important antioxidants and occur at low levels compared with other micromolecular and enzymatic antioxidants (Surai et al. 1997, Hartley and Kennedy 2004). Indeed, the null, nonadaptive hypothesis for the presence of carotenoids in semen is that they simply represent "spillover" from excess carotenoids in circulation. The spillover hypothesis, however, seems unlikely based on observations in other species, including that: (1) carotenoid levels vary across body tissues, suggesting adaptive differential allocation, (2) carotenoid levels in different body tissues respond differently to manipulations (e.g., dietary carotenoid supplementation or

restriction, immune challenges; Koutsos Calvert, and Klasing 2003, Koutsos, Clifford et al. 2003), and (3) excess carotenoids can be voided in waste (McGraw 2006).

An alternative hypothesis is that seminal fluid carotenoids, even in low concentrations, serve an antioxidant function to protect sperm from oxidative damage. The assumptions of this hypothesis are that avian sperm and male fertilizing capacity are negatively affected by free-radical attack, and that carotenoids mitigate such oxidative damage. Avian sperm does appear to be vulnerable to oxidation by free radicals (Surai et al. 1998, 2001) and, at least in domestic fowl, free-radical production is associated with reduced motility and loss of fertilizing potential (Fujihara and Howarth 1978, Wishart 1984). Additionally, the antioxidant properties of carotenoid pigments are relatively well established (Miki 1991, Bendich 1993, but see Costantini and Møller 2008), and it has been suggested that carotenoids play a role in protecting sperm from oxidative stress in other animal taxa (Palan and Naz 1996, Heller et al. 2000, Agarwal et al. 2005).

The presence of carotenoids in seminal fluid suggests that carotenoid-based ornamentation has the potential to signal carotenoid antioxidant defense of sperm and subsequent sperm quality. A key test will be to determine if semen from more colorful individuals contains higher concentrations of carotenoids than that of less colorful individuals and if these molecules influence sperm quantity or quality. If carotenoids do not play a major role in antioxidant defense of sperm, and carotenoid-based traits are correlated with fertilization ability, such traits may instead indicate the availability of alternate antioxidants such as the colorless, noncarotenoid antioxidants vitamins C and E (Hartley and Kennedy 2004, Pike, Blount, Lindström, and Metcalfe 2007). Future studies should test the active role of carotenoids as antioxidants in seminal fluid, alongside the activity of other antioxidants (e.g., vitamins C and E, and glutathione) and antioxidant systems (superoxide dismutase [SOD], glutathione peroxidase [GSH-Px], and metal-binding proteins [e.g., selenium]; Surai et al. 2001).

We found significant differences among species in the types and concentrations of carotenoid pigments in the seminal fluid of fairy-wrens. Lutein and zeaxanthin, present in Superb and Splendid Fairy-Wrens, are common dietary carotenoids and are likely transferred to seminal fluid directly from food through circulation without metabolism. In contrast, we believe Red-backed Fairy-Wrens most likely metabolically derive ketocarotenoids from dietary precursors and subsequently transfer these carotenoids directly from circulation to semen. These differences may, at least partially, be attributable to the phylogenetic relationships of the species examined. In particular, the seminal fluid carotenoid profiles of the Superb and Splendid Fairy-Wrens may be more similar because these species are more closely related to each other than either is to the Red-backed Fairy-Wren (Christidis and Schodde 1997). However, at this time we cannot definitively determine the cause(s) of the interspecific variation in the carotenoid profile of fairy-wren seminal fluid (e.g., variation in type of carotenoid consumed, carotenoid assimilation or metabolism, or phylogeny).

Fairy-wrens exhibit very high levels of extra-pair paternity, and males possess several adaptations associated with intense sperm competition (Rowe and Pruett-Jones 2006, Rowe et al. 2008). For the species examined in this study and based on rates of extra-pair paternity, the intensity of sperm competition appears to be greatest in the Superb Fairy-Wren, followed by the Red-backed and then the Splendid Fairy-Wren (Mulder et al. 1994, Dunn and Cockburn 1999, Karubian 2002, Webster et al. 2004, Webster et al. 2008). The seminal fluid carotenoid profile of Red-backed Fairy-Wrens is particularly noteworthy because males display red, carotenoid-based plumage coloration, and ketocarotenoids were common to the three tissue samples analyzed: sperm, plasma, and feathers. Ketocarotenoids are often regarded as more potent antioxidants relative to the xanthophylls (Miki 1991) and, as such, may offer a greater degree of sperm protection and be more effective at maintaining the fertilizing capacity of a male's sperm. The occurrence of ketocarotenoids in the seminal fluid of the Red-backed but not the Superb Fairy-Wren suggests that carotenoid type is not associated with sperm competition intensity. At least qualitatively, however, the pattern of interspecific variation in total carotenoid concentration mimics the pattern of variation in sperm competition intensity. The correspondence of these patterns suggests that seminal fluid carotenoid concentrations may have evolved in response to selective pressures resulting from sperm competition. More specifically, defense from oxidative stress awarded sperm by carotenoids (and perhaps noncarotenoid antioxidants) may function to increase a male's likelihood of paternity success under conditions of sperm competition by increasing the relative fertilizing capacity of his sperm.

In summary, the presence of carotenoids in the seminal fluid of fairy-wrens suggests that, although carotenoids may have limited antioxidant action in the seminal fluid of these species (and likely others) due to their low concentrations, there appears to be interspecific variation in avian sperm carotenoids on which selection could act. The identification of carotenoids in avian seminal fluid validates a key assumption underlying the hypothesis that carotenoids provide a mechanistic link between male ornamentation and fertilizing capacity. Moreover, this work suggests that carotenoid-based plumage has the potential to reveal information regarding the carotenoid content, and possibly antioxidant defenses, of semen and thus advertise male fertilizing capacity. While this work is preliminary, it suggests that further examination of seminal fluid carotenoids and their role in both sperm antioxidant defense and sexual signaling will be an important area of future research.

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APPENDIX. Diagnostic characteristics of carotenoids present in the seminal fluid, plasma, or feathers of fairy wrens, as determined using high-performance liquid chromatography (HPLC) and UV-visible detection. RT = retention time (min), λ_{max} = wavelength(s) of maximum absorbance (nm).

Pigment	RT	λ_{max}
Canary xanthophyll B	4.9	443, 471
Canary xanthophyll A	5.1	443, 471
Adonirubin	5.5	474
Alpha-doradexanthin	6.3	479
Lutein	6.6	448, 476
Astaxanthin	7.6	478
Zeaxanthin	7.9	454, 481
Canthaxanthin	9.3	476