



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS  
BIOLÓGICAS

Instituto de Ecología

ESTRUCTURA Y FUNCIÓN DEL CANTO DEL  
CARDENALITO (*Pyrocephalus rubinus*)

TESIS

QUE PARA OBTENER EL GRADO ACADÉMICO DE  
DOCTOR EN CIENCIAS

PRESENTA

ALEJANDRO ARIEL RÍOS CHELÉN

DIRECTOR DE TESIS: DR. CONSTANTINO MACÍAS GARCÍA

MÉXICO, D.F.

MARZO, 2006



COORDINACIÓN



Universidad Nacional  
Autónoma de México

Dirección General de Bibliotecas de la UNAM

**Biblioteca Central**



**UNAM – Dirección General de Bibliotecas**  
**Tesis Digitales**  
**Restricciones de uso**

**DERECHOS RESERVADOS ©**  
**PROHIBIDA SU REPRODUCCIÓN TOTAL O PARCIAL**

Todo el material contenido en esta tesis esta protegido por la Ley Federal del Derecho de Autor (LFDA) de los Estados Unidos Mexicanos (México).

El uso de imágenes, fragmentos de videos, y demás material que sea objeto de protección de los derechos de autor, será exclusivamente para fines educativos e informativos y deberá citar la fuente donde la obtuvo mencionando el autor o autores. Cualquier uso distinto como el lucro, reproducción, edición o modificación, será perseguido y sancionado por el respectivo titular de los Derechos de Autor.

Ing. Leopoldo Silva Gutiérrez  
Director General de Administración Escolar, UNAM  
Presente

Por medio de la presente me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 26 de septiembre del 2005, se acordó poner a su consideración el siguiente jurado para el examen de DOCTOR EN CIENCIAS del alumno **RÍOS CHELÉN ALEJANDRO ARIEL** con número de cuenta **93770583**, con la tesis titulada: "Estructura y función del canto del cardenalito (*Pyrocephalus rubinus*)", bajo la dirección del Dr. **Constantino Macías García**.

Presidente:	Dr. Jesús Alejandro Estrada Medina
Vocal:	Dra. Katherine Renton
Vocal:	Dra. B. Patricia Escalante Pilego
Vocal:	Dra. Blanca Estela Hernández Baños
Secretario:	Dr. Constantino Macías García
Suplente:	Dra. Roxana Torres Áviles
Suplente:	Dra. María Marcela Osorio Beristain

Sin otro particular, quedo de usted.

Atentamente  
"POR MI RAZA HABLARÁ EL ESPÍRITU"  
Ciudad Universitaria D.F., a 30 de noviembre del 2005



Dr. Juan Núñez Farfán  
Coordinador del Programa

c.c.p. Expediente del interesado

Agradezco el apoyo otorgado por CONACYT y por PAEP (proyecto # 201318) para la realización de esta investigación

Agradezco también a los miembros de mi comité tutorial:

Dr. Constantino Macías Garcia

Dr. Carlos Cordero

Dra. Katherine Renton

por su tiempo y valiosa retroalimentación

**Estructura y función  
del canto del cardenalito  
*Pyrocephalus rubinus***

A mis abuelos  
A mis padres

# **Agradecimientos**

## AGRADECIMIENTOS

Muchas gracias a Constantino, director de esta tesis, por haberme apoyado tanto en este proyecto de doctorado. Esto implica no sólo su apoyo en México sino también aquel para que pudiera realizar la estancia de investigación en Holanda.

Gracias a mis tutores Carlos Cordero y Katherine Renton que, con su oportuna retroalimentación, dieron buenos consejos y útiles opiniones sobre diversos aspectos de esta tesis.

Gracias a mis sinodales por sus comentarios y por firmarme rápidamente los votos aprobatorios: Constantino Macías, Katherine Renton, Roxana Tórres, Marcela Osorio, Blanca Hernández, Patrica Escalante y Alejandro Estrada.

Gracias a Hugh Drummond por haber formado este laboratorio.

Gracias a todos los que de alguna u otra forma compartieron conmigo el tiempo que duró este proyecto, miembros (de ahora o de antes) del laboratorio de conducta animal del Instituto de Ecología.

Many thanks to all members (and former members) of the Evolutionary Biology Group at Leiden University: Carel ten Cate, Katharina Riebel, Albertine Leitão, Selvino de Kort, Gábríel Beckers and Rob Lachan among others. Especial thanks to Katharina who taught me to think in "all other possibilities" and "destroy" my work before anyone else does. Thanks to Nicole Geberzahn, Emma Villaseñor and Adriana Vallarino for your help with the statistics. Thanks to Dietmar Todt for your time and kind willingness to talk with me, to Peter Slater for your accurate and appropriate comments, and to WJ Smith for your feedback and for sharing your opinion with us (Constantino, Katharina and me).

Así como a otras personas muy especiales que compartieron mi casa y mi vida desde que empecé la tesis hasta ahora. Algunos de ellos estudiantes de doctorado y postdocs.

A Anahí también por estar aquí.

A Lilia y Carolina por ayudar (o haber ayudado) a hacer del Posgrado en Ciencias Biológicas un lugar más ameno y eficiente, a pesar de todas las cartas, firmas, papeles y más firmas que hay que entregar semestre tras semestre.

Gracias a Margarita Martínez y Rosa A. Lucio, por apoyarnos tanto con las instalaciones del Centro Tlaxcala de Biología de la Conducta en Tlaxcala, durante el trabajo de campo. ¡Muchas gracias a los miembros y estudiantes o ex estudiantes de este Centro!

En fin, gracias a todos los que me han ayudado y no menciono pero que están o estuvieron ahí: ayer, ahora y mañana.



# Contenido

## **Resumen / Abstract**

Capítulo 1

**Sobre la función del canto en las aves**

Capítulo 2

**Variation in the song of a sub-oscine,  
the vermilion flycatcher**

Capítulo 3

**Flight display song of the  
vermilion flycatcher**

Capítulo 4

**Effects of song length on territorial  
responses during song playback  
in a sub-oscine bird**

Capítulo 5

**Extra-pair reproduction  
and song length in the  
vermilion flycatcher**

Capítulo 6

**Conclusiones generales**

**Agradecimientos**

# **Resumen / Abstract**

## RESUMEN

Se estudió la función adaptativa de los cantos del cardenalito (*Pyrocephalus rubinus*), y en especial este estudio se enfocó a la selección intra-sexual. Se investigó cómo algunos atributos del canto (e.g. longitud del canto), pueden transmitir diferentes mensajes (e.g. identidad de individuo, disposición de atacar) a otros machos, y también si estos atributos se relacionan o no con el éxito reproductivo.

Se demostró que el canto de los cardenalitos varía intra-individualmente en atributos relacionados con la longitud del canto (e.g. número de elementos introductorios). Los machos cantan más y utilizan cantos más largos cuando la hembra ha comenzado la construcción del nido que en el periodo previo a la construcción del nido. Esta variación en el canto no contrarrestó la variación inter-individual también encontrada en la longitud del canto. Por otro lado, atributos relacionados con la segunda parte del canto (e.g. la longitud del elemento terminal) mostraron los mayores valores de repetibilidad, sugiriendo que podrían conferir información sobre identidad individual.

Estos resultados nos llevaron a abordar la pregunta de si esta variación en el canto significa algo para los machos. Se investigó si los machos son capaces de discriminar entre variaciones en longitud del canto y en la tasa de canto, y cómo estas variaciones podrían ser usadas para comunicar diferentes mensajes en diferentes momentos de la temporada reproductiva. Para abordar estas preguntas, hicimos experimentos de playback y encontramos que los machos vocalizan más cuando son expuestos a cantos largos que cuando escuchan cantos cortos. Esto fue así antes de la construcción del nido, pero no después. Estos resultados sugieren que los cantos largos son percibidos como señales más amenazantes que los cantos cortos y que la sensibilidad de los machos a los cantos cambia con la temporada reproductiva.

Determinamos si la variación inter- e intra-individual en los cantos y la capacidad de discriminación de los machos entre cantos largos y cortos están relacionadas con una posible función de los cantos como una señal de resguardo de paternidad. Análisis con microsatélites revelaron que 16-25% de las familias estudiadas presentaron paternidad extra-pareja. Encontramos también casos de quasi-parasitismo y parasitismo intra-específico. Para probar la hipótesis de que los cantos son usados como una señal de resguardo de paternidad, se evaluó la posibilidad de que los machos que utilizan cantos más largos sufran menos pérdida de paternidad en su nido, además de ser padres de una mayor proporción de pollos. No encontramos ninguna relación entre la longitud del canto y el éxito reproductivo de los machos o la cantidad de paternidad perdida. Sin embargo, encontramos que, por medio de la paternidad extra-pareja, la varianza en éxito reproductivo entre machos se podría estar incrementando hasta 1.6-2.7 veces por arriba de la que se esperaría en ausencia de paternidad extra-pareja. Esto sugiere que la paternidad extra-pareja podría jugar un papel importante en la evolución

del dimorfismo sexual en esta especie. Sin embargo, discutimos algunos puntos por los que estos resultados deben tomarse con cautela.

En conclusión, se demostró que las variaciones naturales en el canto no son pasadas por alto por los cardenalitos, ya que los cantos más largos parecen ser percibidos como señales más amenazantes, pero también se aportó evidencia (no concluyente) de que a través de la paternidad extra-pareja los machos pueden incrementar la varianza en éxito reproductivo. Este trabajo abre las puertas a investigaciones futuras para determinar qué aspectos (i.e. cantos, color, cortejo) se relacionan con la conducta extra-pareja en esta especie.

## ABSTRACT

We studied the adaptive function of the song of the vermilion flycatcher (*Pyrocephalus rubinus*), focusing on intra-sexual selection. We aimed to determine how song attributes (e.g. song length) might convey different messages (e.g. individual identity, willingness to attack) to other males, and whether or not these attributes relate to reproductive success.

We demonstrate that vermilion flycatcher males show significant intra-individual variation in song attributes related to song length (e.g. number of introductory elements). Males sing more and longer songs when females have commenced nest construction than prior to the onset of nest construction. This nest cycle variation in song delivery did not outweigh the inter-individual variation also found in song length. On the other hand, attributes related to the second part of the song (e.g. terminal element length) showed the greatest repeatability values, suggesting a possible function for individual identity.

These results led us to tackle the question of whether or not the observed variation in song delivery is meaningful to males. We asked whether males are able to discriminate between variations of song length and song rate, and how song variants may be used to communicate different messages in different periods of the nest cycle. We performed playback experiments and found that males vocalized more in response to long than to shorter songs. This was the case before the onset of nest construction, but not after this period. These results suggest that long songs are perceived as more threatening signals than short songs and that male sensitivity to songs changes with the breeding cycle.

We also determined whether the inter- and intra-individual variation in song delivery, and the fact that males are able to discriminate between long and short songs, were related to a possible function of song as a paternity guarding signal. Micro-satellites analyses revealed extra-pair paternity in 16-25% families, as well as cases of quasi-parasitism and intra-specific brood parasitism. To test the hypothesis that songs are used as a paternity guarding signal, we evaluated whether males singing longer songs maintain higher paternity and sire a higher proportion of young than males singing short songs. We did not find any significant correlation between song length and male reproductive success and/or

intensity of lost paternity. We found that, through extra-pair paternity, variance in male reproductive success might increase to 1.6-2.7 times over that that would be expected in the absence of extra-pair paternity. This suggests that extra-pair paternity could be playing an important role in the evolution of sexual dimorphism in this species. However, since these results have some problematic points, they should be taken with caution.

In conclusion, we demonstrated that natural song variation is meaningful for vermilion flycatcher males, as longer songs seem to be perceived as more threatening signals, but also provide (inconclusive) evidence that through extra-pair paternity males can increase the variance in reproductive success. This study opens the door for future research regarding what aspects (e.g. song, colour, courtship) are related to extra-pair behaviour in this species.

**1**

**Sobre la  
función del  
canto  
en las aves**

# Sobre la función del canto en las aves

Alejandro Ariel Ríos Chelén

“..., realmente podemos sólo explicar la función presente (la utilidad actual) y nunca podemos estar seguros sobre los verdaderos orígenes evolutivos”. Catchpole y Slater (1985)

El canto en las aves se da, con excepciones de aves que habitan principalmente en los trópicos, sólo en los machos y se ha pensado que cumple principalmente dos funciones: atraer hembras y defender el territorio de otros machos.

La variedad de los cantos en las aves es enorme. El repertorio de las aves, es decir el número de diferentes cantos o elementos que canta un macho, varía mucho dependiendo de la especie. Así tenemos que hay especies como el gorrión coroniblanco (*Zonotrichia leucophrys*) que sólo canta un tipo de canto (Baptista 1975), mientras que otros como el zacatonero de cinco-rayas (*Aimophila quinquestriata*) tiene entre 159 y 237 tipos de cantos (Groschupf y Mills 1982), por no mencionar al cuitlacoche rojizo (*Toxostoma rufum*) que llega a tener 2000 tipos diferentes de cantos (Kroodsma y Parker 1977). ¿Cómo se ha originado esta variedad de cantos en la naturaleza? y ¿cómo evoluciona esta diversidad?, son algunas de las preguntas que tratan de responder los ecólogos conductuales que trabajan con cantos.

La función del canto está íntimamente relacionada con la evolución del mismo ya que se piensa, y hay evidencia de que así es, que el canto puede haber evolucionado en gran medida a través de selección sexual. En otras palabras, el canto, así como otras características vistosas en las aves (y otros animales), puede estar evolucionando (cambiando a través de las generaciones) y siendo mantenido en una población como resultado de un proceso que incluye la reproducción diferencial entre individuos. Esta reproducción diferencial puede originarse al atraer más hembras (selección intersexual) o cuando los machos compiten (selección intrasexual) por el recurso que en nuestro caso son las hembras. Cualquiera de los dos mecanismos, o una combinación de ambos, puede ocasionar que ciertos individuos se reproduzcan más que otros dando como resultado que algunas de sus características se expresen en la siguiente generación.

Searcy y Andersson (1986), refiriéndose al canto de las aves, propusieron que para dar evidencia fuerte de que la selección intersexual ocurre es necesario tomar en cuenta dos criterios: 1) las hembras responden de manera diferencial a ciertos atributos del canto cuando ellas son expuestas a los mismos en ausencia del macho, y 2) el mismo atributo del canto se correlaciona con el éxito reproductivo del macho cuando otras



características que pueden influir en la elección de la hembra son controladas. De la misma manera, propuso que para apoyar fuertemente la idea de que la selección intrasexual ocurre es necesario cumplir dos criterios: 1) demostrar experimentalmente que el canto afecta el éxito en la competencia entre machos, y 2) demostrar que el éxito reproductivo se correlaciona ya sea con el éxito en la competencia o con el atributo del canto de interés.

En este trabajo se pretende hacer una revisión de los estudios concernientes a la función del canto en las aves, de los trabajos que dan evidencia o no de la existencia de selección intra e intersexual.

### ELECCIÓN DE LA HEMBRA (SELECCIÓN INTERSEXUAL)

Eriksson y Wallin (1986), estudiando dos especies de papamoscas (*Ficedula hypoleuca* y *F. albicollis*), obtuvieron clara evidencia de que el canto atrae a las hembras. Ellos pusieron altavoces y machos disecados cerca de cajas nido que contenían trampas y encontraron que más hembras fueron atrapadas en los nidos con grabaciones que en aquellos sin grabaciones. Por otro lado, en condiciones seminaturales (en aviario) West et al (1981a y b) observaron que los machos dominantes del vaquero cabecicafé (*Molothrus ater*) obtienen más cópulas que los machos subordinados, y, aunque las hembras no responden más a grabaciones de cantos de machos dominantes que de cantos de machos subordinados, sí necesitan que el macho cante para aceptar copular. Parece ser que el éxito reproductivo de los machos dominantes se debe, al menos en parte, a las preferencias de la hembra por ciertos atributos del canto que distingue a los machos dominantes.

Catchpole (1980) trabajando con la especie *Acrocephalus schoenobaenus* mostró que los machos con repertorios grandes atraen pareja antes de la temporada de reproducción que machos con repertorios pequeños. Cuatro años después, Catchpole et al (1984) mostraron en estudios de laboratorio que las hembras de esta misma especie responden más fuertemente a grabaciones de grandes repertorios que de pequeños repertorios.

Los machos de la grulla (*Centrocercus urophasianus*) no cantan pero producen, durante su despliegue en leks, sonidos como “silbidos” y “chasquidos”. Los machos con mayor éxito reproductivo son los que tienen un periodo más largo de frecuencia ascendente y un pico de frecuencia mayor en la porción del “silbido” de su despliegue de cortejo (Gibson y Bradbury 1985). Este éxito reproductivo diferencial bien podría deberse

a que las hembras son atraídas por los machos con estas características en las vocalizaciones que emiten. Gibson (1989) llevó a cabo experimentos de playback usando grabaciones para aumentar los despliegues naturales y encontró que más hembras eran atraídas al lek en los días en que estaba la grabación.

Hay varios estudios más que satisfacen el criterio 1 de Searcy y Andersson. Por ejemplo en canarios (*Serinus canarius*) cautivos, las hembras realizan más conducta de construcción de nido cuando son expuestas a repertorios grandes (Kroodsma 1976 en Searcy y Andersson 1986). Las preferencias de las hembras por ciertos cantos podrían relacionarse con preferencias por machos de alta calidad. Drăgănoiu et al. (2002) mostraron que las hembras de canarios (*Serinus canaria*) prefieren atributos del canto que no se encuentran en la naturaleza, en particular cantos que combinan una alta tasa de producción de elementos más una amplia frecuencia de producción de dichos elementos. Ya que existen limitante motoras para que un macho produzca este tipo de cantos de forma natural, Drăgănoiu et al. (2002) han sugerido que las preferencias de las hembras por estos cantos podrían indicar una preferencia "latente" por machos, que de producir estos cantos, serían de alta calidad. Por otro lado, las hembras del gorrión pantanero (*Melospiza georgiana*) cortejan más cuando escuchan grabaciones de cantos múltiples que al escuchar grabaciones con un solo tipo de canto (Searcy et al 1982). Otros estudios indican que las preferencias de las hembras por ciertos cantos podrían relacionarse con información sobre la identidad del individuo que estos cantos podrían transmitir. Wiley et al. (1991) estudiaron parejas ya formadas de la especie *Prunella modularis*. Quitaron al macho y observaron que las hembras respondían al playback pero sólo durante su periodo fértil. Durante este periodo, las hembras se acercaron a grabaciones de su propio macho en preferencia de grabaciones de sus vecinos.

En especies que carecen de grandes repertorios, la información en los cantos podría estar contenida en variaciones en la tasa del canto o en la longitud del mismo. Wasserman y Cigliano (1991) encontraron que las hembras del gorrión gorjiblanco (*Zonotrichia albicollis*) responden con mas despliegues copulatorios ante tasas de canto mayores y cantos de mayor longitud (con más elementos). Por otro lado, Alatalo et al (1990) controlaron experimentalmente los posibles efectos que podría tener la calidad del territorio en atraer hembras y manipularon experimentalmente la tasa de canto de una especie de papamoscas (*Ficedula hypoleuca*) aumentando la cantidad de comida en algunos territorios ocupados. La tasa de canto de este papamoscas disminuye a bajas temperaturas pero un incremento artificial en la alimentación puede aumentar la tasa de

canto al nivel normal que se espera en buenas condiciones de tiempo (Gottlander 1987). Alatalo et al (1990) encontraron que los machos experimentales fueron más exitosos en atraer hembras y esto se debió probablemente al incremento en la tasas de canto. También, Hoi-Leitner et al (1995) y Radesäter et al. (1987) obtuvieron evidencia que sugiere que las hembras de las especies *Sylvia atricapilla* y *Phylloscopus trochilus* respectivamente usan la tasa de canto para tomar decisiones sobre apareamiento. Por otro lado, los estudios llevados a cabo por Martin-Vivaldi et al. (1999) muestran que las hembras de la especie *Upupa epops* obtienen beneficios directos al preferir machos que utilizan cantos con estrofas más largas. Estos machos traen más comida a los pollos y tienen un mayor éxito reproductivo. De manera que los estudios de Martin-Vivaldi y colaboradores apoyan los dos criterios de Searcy y Andersson para evaluar selección sexual.

Los resultados en los estudios sobre selección sexual pueden variar a través del tiempo, incluso dentro de una misma especie. Gil y Slater (2000), estudiando a la especie *Phylloscopus trochilus* por un periodo de 4 años, encontraron que los machos con repertorios grandes obtuvieron pareja antes que los machos con repertorios pequeños. Sin embargo esta correlación sólo ocurrió durante uno de los cuatro años de estudio, en los demás años no hubo correlación entre ninguna característica del canto y la fecha de obtención de pareja. Por otro lado, los machos con repertorios grandes tuvieron más pollos que llegaron a emplumar. Concluyeron que en la mayoría de los años no hay selección sexual por preferencias de la hembra sobre alguna característica del canto, sin embargo los datos de éxito reproductivo apoyan la idea de que el tamaño de repertorio puede ser un indicador de calidad del macho.

Como ya se ha hecho mención, un problema serio al tratar de demostrar que las hembras escogen a un macho basándose en cierta característica (p.e. tamaño de repertorio) es que esa característica podría estar correlacionada con la calidad del territorio, y es posible que la hembra se aparee con un macho no porque tiene esa característica sino porque dicho macho se encuentra en un territorio de alta calidad (p.e. con mucha comida). Para solucionar este problema es necesario hacer un estudio en donde se controle experimentalmente la calidad del territorio, o bien controlar esta variable con métodos estadísticos. Mountjoy y Lemon (1996) mostraron que los machos del estornino europeo (*Sturnus vulgaris*) con cantos más complejos adquirieron pareja más rápido. Esta relación se mantuvo aún cuando controlaron estadísticamente la preferencia por territorios. Esto muestra que las hembras de esta especie se basan en la

complejidad del canto, más que en la calidad del territorio, para escoger a su pareja. Además los machos con repertorios grandes se encontraban en mejor estado físico, indicando que las hembras obtienen machos de alta calidad al escogerlos basándose en el canto. Otros estudios muestran también una clara correlación entre canto y estado físico del macho. Por ejemplo, Buchanan et al (1999) encontraron que en la especie *Acrocephalus schoenobaenus*, el tamaño del repertorio de los machos con parásitos (*Haemoproteus* sp, *Trypanosoma* sp y *Plasmodium* sp) es menor al de los machos no parasitados. Estos autores sugirieron que las infecciones pueden reducir la expresión de características del canto sujetas a selección sexual. Por otro lado, Hasselquist et al. (1996) demostraron que las hembras de la especie *Acrocephalus rubinus* buscan cópulas extra-pareja de vecinos que utilizan repertorios de cantos más grandes que sus parejas. Los pollos de los padres genéticos con repertorios más grandes tuvieron una mayor probabilidad de sobrevivir que los pollos de padres con repertorios más pequeños, sugiriendo que las hembras buscan beneficios genéticos para sus crías en machos extra-pareja con repertorios más grandes. Estos estudios sugieren que el tamaño de repertorio puede dar información sobre la calidad del macho (i.e. estado de salud) y que las hembras que busquen parejas que usan repertorios grandes pueden beneficiarse al incrementar la viabilidad de sus hijos.

### COMPETENCIA ENTRE MACHOS (SELECCIÓN INTRASEXUAL)

Los experimentos que involucran manipular físicamente a los machos para impedir que canten han dado una clara evidencia de que el canto influye en las contiendas entre machos. Peek (1972) fue pionero en estos experimentos. Trabajando con el tordo sargento (*Agelaius phoeniceus*), Peek anestesió varios machos y les sacó una pequeña porción del nervio hipoglosal que enerva la siringe, logrando así que estos machos no cantaran. Cuando los machos se recuperaron perfectamente los reintrodujo a sus territorios y pudo comprobar que los territorios de estos machos fueron invadidos por otros machos mucho más frecuentemente que los territorios de machos que sí podían cantar.

Smith (1979) utilizó una técnica diferente para impedir que un macho cante. En vez de alterar los nervios hipoglosales, él perforó la membrana del saco aéreo interclavicular del tordo sargento. Una de las ventajas de esta operación es que los machos pueden recobrar el canto una vez que la membrana se cura. Con esta técnica obtuvo resultados semejantes a los obtenidos anteriormente por Peek. Utilizando esta misma técnica, McDonald (1989) estudió el gorrión marino (*Ammodramus maritimus*). Con este

experimento McDonald demostró que los machos que no podían cantar eran más lentos para obtener un territorio y sufrieron más invasiones de sus vecinos. Algo interesante es que en este caso, los gorriones no perdieron la facultad de emitir otro tipo de vocalizaciones, como llamados. Por esto, este experimento es una prueba más contundente de que el canto por sí sólo (sin incluir llamados) funciona para defender el territorio.

Göransson et al. (1974) fueron pioneros en hacer otro tipo de enfoque. Ellos realizaron experimentos en donde un macho en su territorio es reemplazado por una grabación. Este método tiene la ventaja de que cualquier otra variable que pueda tener el macho, y que afecte la defensa del territorio, es controlada al quitar dicho macho y los resultados obtenidos son, con mayor certeza, debidos a un efecto de la variable "canto". Göransson et al. (1974) estudiaron una especie del género *Luscinia* (*Luscinia luscinia*) y mostraron que los territorios vacíos pero con grabaciones tardaban más tiempo en ser ocupados que los territorios sin grabaciones. Tratando de vislumbrar los efectos del tamaño del repertorio, Krebs et al (1978) estudiaron una especie de paro (*Parus major*), y mostraron que los territorios de esta especie que estaban "defendidos" por una grabación de un repertorio grande permanecían más tiempo sin ser ocupados que los territorios con grabaciones de repertorios pequeños. Relacionado con esto, Hiebert et al (1989) encontraron que los machos del gorrión cantor (*Melospiza melodia*) que tienen repertorios más grandes pudieron mantener más tiempo sus territorios y tuvieron mayor éxito reproductivo, tanto en el lapso de un año como en el transcurso de toda la vida.

Los estudios sobre vocalizaciones y selección intrasexual en aves no se limitan a especies que cantan. Las interacciones con vocalizaciones entre machos ocurren también en aves no cantoras (no cantoras). Por ejemplo, Slabbekoorn y ten Cate (1998) han estudiado los llamados ("coos") emitidos por palomas. Ellos trabajaron con la especie *Streptopelia decaocto* y encontraron que los "coos" modulados originaron una respuesta mayor en machos que los "coos" no modulados, y pudieron determinar que el rango de frecuencia modulada que originaba una mayor respuesta en los machos era de 75-108 Hz, aún cuando frecuencias mayores todavía se encuentran dentro del rango natural de la especie.

Hay otros estudios que se relacionan no sólo con los cantos o con los llamados, sino con ambas vocalizaciones. Adhikerana y Slater (1993) estudiaron una especie de paro (*Parus ater*) y usando experimentos de playback encontraron que las grabaciones con una tasa de canto que aumentaba inhibieron los cantos de las aves que respondían

pero, por otro lado, las incitaron a producir más llamados. También encontraron que las grabaciones de cantos más largos indujeron más llamados en respuesta que los cantos cortos. Concluyeron que los cantos con una tasa que aumenta origina una respuesta en los machos como si se tratara de una amenaza más seria que los cantos con una tasa que disminuye, y por otro lado, que los cantos más largos producen también una respuesta como si se tratara de una amenaza más seria que cantos más cortos.

Como hemos visto, varios estudios han tratado de entender cómo interactúan los machos ante cantos de su misma especie. Estas interacciones pueden estar relacionadas con la competencia por un territorio y en última instancia por las hembras. Sin embargo, las interacciones entre machos para ocupar un territorio también pueden darse entre dos especies diferentes. Gil (1997) estudió dos especies del mismo género (*Certhia brachydactyla* y *C. familiaris*) que, dentro de un rango altitudinal, viven tanto en simpatria como en alopatría. Haciendo experimentos de playback, encontró que los machos de *C. brachydactyla* en simpatria mostraron una respuesta más agresiva hacia el canto de *C. familiaris* que aquellos en alopatría. Aunque no encontró territorialidad interespecífica, los datos sugieren que el patrón de distribución altitudinal de estas dos especies puede ser una consecuencia del incremento en la agresión. Por otro lado, Doutrelant et al. (2000), trabajando con *Parus caeruleus*, encontraron que la estructura del canto en esta especie podría haber evolucionado para disminuir las interacciones agonísticas con un competidor cercano, la especie *Parus major*. En particular, la producción de ciertos elementos al final de los cantos de *Parus caeruleus* parece disminuir las probabilidades de que esta especie sea considerada como competidora por *Parus major*: la respuesta ante playback con estos cantos es menor que cuando los cantos no tienen estos los elementos finales.

Otros estudios se relacionan con dos fenómenos conocidos como ajuste de canto (song matching) y ajuste de repertorio (repertoire matching). Ajuste de canto se refiere a que cuando un macho canta, otro macho le responde utilizando el mismo canto. Ajuste de repertorio se refiere a que cuando un macho canta, su vecino le responde usando un canto que ambos comparten en su repertorio. Beecher et al (1996), estudiando las interacciones en el gorrión cantor, descubrieron que esta ave responde selectivamente al canto de un vecino. Selecciona uno de los cantos que comparte con ese vecino para responder. Estos investigadores dieron evidencia de que un gorrión no responde a un vecino con el mismo canto de éste, aún cuando tenga en su repertorio dicho canto. En cambio, responde con un ajuste de repertorio. Sin embargo, en ese y otros estudios se ha visto que cuando se trata del canto de un extraño (no vecino), sí responde con el mismo

canto (ajuste de canto). Esto se ha interpretado como que el macho que responde percibe el canto de un extraño como más amenazante que el canto de un vecino que, por otro lado, puede representar un competidor más familiar. Para apoyar esta hipótesis y examinar la posibilidad de que ajuste de canto es una respuesta más fuerte que ajuste de repertorio, algunos de estos autores hicieron otros experimentos de playback y encontraron que, en la época en que se están estableciendo los territorios, los machos responden a una grabación con ajuste de canto, mientras que responden a esta misma grabación con ajuste de repertorio cuando la temporada de establecimiento de territorio ya ha pasado (datos no publicados, en Beecher et al 1996). Se ha pensado que responder al canto de un vecino con el mismo canto es una manera particularmente efectiva de desanimar a un intruso.

## OTROS ESTUDIOS

En los estudios presentados anteriormente no se cuestiona la posibilidad de que machos y hembras perciban los cantos de manera diferente y de las implicaciones que esto podría tener en el proceso de selección sexual. Hay evidencia de que machos y hembras pueden percibir los cantos de manera diferente (Ikebuchi et al. 2003) y que los cantos podrían estar sujetos a presiones de selección contrarias. Es decir que mientras las hembras prefieren ciertos atributos, los cantos con atributos contrarios podrían ser más efectivos para repeler intrusos. Por ejemplo, los cantos de la especie *Fringilla coelebs*, tienen en su parte final un “adorno” de elementos que varía en longitud. Leitão y Riebel (2003) mostraron que mientras los cantos con “adornos” más cortos parecen ser señales más amenazantes para los machos, los cantos con “adornos” más largos son preferidos por las hembras. De esta manera, parece que no siempre la selección sexual intra e intersexual apuntan a un mismo camino.

Otros estudios que han adquirido gran interés en los últimos años se relacionan con la posibilidad de que la comunicación no se de únicamente entre el ave que canta y el ave al que está dirigido el canto. Por ejemplo, los estudios de Mennill et al. (2002) muestran que las hembras podrían estar “espiando” los cantos durante las interacciones agresivas entre machos para decidir con qué macho aparearse. Por otro lado, Tobias y Seddon (2002) sugieren que los machos también podrían “espíar” las vocalizaciones entre parejas para obtener información sobre la localidad y estado fértil de la hembra y así obtener cópulas extrapareja. Por último, también hay evidencia de que los machos, al escuchar las contiendas entre otros machos, pueden obtener información sobre quien es

ganador y quien es perdedor, y que esta información puede influir en cómo responden en sus futuras interacciones intrasexuales (Peake et al. 2002).

## CONCLUYENDO

Hay evidencia inequívoca de que el canto atrae hembras y de que puede producir en ellas conductas relacionadas con la reproducción. Igualmente hay evidencia de que el canto sirve para repeler machos del territorio y que puede producir en machos vecinos conductas asociadas a la competencia por un territorio. Esto es importante porque en muchas especies, el tener un territorio es un requisito para optar a una hembra. Varios estudios se han enfocado a uno u otro aspecto de la selección sexual, es decir al efecto que tienen las vocalizaciones de los machos en las hembras o al efecto que tienen sobre otros machos. En este sentido sería interesante hacer estudios relacionados con ambos aspectos para intentar vislumbrar, dentro de una misma especie, la importancia relativa que tiene el canto en cada uno de ellos.

Por otro lado, aunque no todas, varias de las investigaciones relacionadas con selección sexual y canto se han enfocado sólo a uno de los dos criterios expuestos por Searcy y Andersson (1984) (tabla 1 y 2). Es de gran importancia tratar de abordar ambos criterios, es decir estudiar los efectos del canto en las hembras y en los machos y por otro lado investigar el éxito reproductivo que pueda asociarse a un atributo particular del canto.



Tabla 1. Estudios que relacionan: éxito reproductivo con algún atributo del canto o vocalización y una respuesta diferencial de la hembra con alguna característica del canto. También se anotan estudios donde se da evidencia de ambos fenómenos en una misma especie.

Especie	1) Éxito reproductivo	2) Respuesta diferencial de la hembra que incluye atracción de pareja antes en la temporada	Ambos fenómenos (1 y 2)	Referencia
Papamoscas <i>Ficedula hypoleuca</i> y <i>F. albicollis</i>		sí		Eriksson y Wallin 1986
<i>Acrocephalus schoenobaenus</i> vaquero cabecicafé <i>Molothrus ater</i>			sí  El éxito reproductivo podría deberse en parte a preferencias de la hembra por ciertos atributos del canto	Catchpole 1980 y Catchpole et al 1984 West et al 1981a y b
grulla <i>Centrocercus urophasianus</i>			Sí	Gibson y Bradbury 1985 y Gibson 1989
Canario <i>Serinus canarius</i>		sí		Kroodsma 1976
Gorrion pantanero <i>Melospiza georgiana</i>		sí		Searcy et al 1982
Gorrion coroniblanco		sí		Baker 1983, Baker et al 1981 y Baker et al 1982.
<i>Prunella modularis</i>		sí		Willey et al 1991
<i>Phylloscopus trochilus</i>	sí	sí aunque sólo en un año de estudio	sí en un año	Gil y Slater 2000
<i>Ficedula hypoleuca</i>		sí		Alatalo et al 1990
Gorrion gorjiblanco ( <i>Zonotrichia albicollis</i> )		sí		Wasserman y Cigliano 1991
<i>Sylvia atricapilla</i>			sí	Hoi-Leitner et al 1995
Estornino europeo <i>Sturnus vulgaris</i>		sí		Mounjoy y Lemon 1996

Tabla 2. Estudios que relacionan 1) éxito reproductivo con algún atributo del canto y 2) alguna característica del canto con el resultado en la competencia por el territorio. En el punto 2 también se consideran estudios que relacionan el canto con una respuesta diferencial del macho. También se ponen estudios que evidencian la presencia de ambos procesos (1 y 2) en una misma especie

Especie	1)Éxito reproductivo	2)Influencia del canto en el resultado de la competencia por el territorio	Ambos fenómenos (1y 2) en una misma especie	Referencia
Tordo sargento <i>Agelaius phoeniceus</i>		sí		Peek 1972
Tordo sargento <i>Agelaius phoeniceus</i>		sí		Smith 1979
Gorrión marino <i>Ammodramus maritimus</i> <i>Luscinia luscinia</i>		sí		McDonald 1989
<i>Parus major</i> Gorrión cantor <i>Melospiza melodia</i>		sí	sí	Göransson et al 1974 Krebs et al 1978 Hiebert et al 1989
Paloma <i>Streptopelia decaocto</i> <i>Parus ater</i>		sí		Slabbekoorn y ten Cate 1998
<i>Certhia brachydactyla</i> y <i>C. familiaris</i> Gorrión cantor		sí		Adhikerana y Slater 1993 Gil 1997
		sí		Beecher et al 1996

Como hemos visto, los estudios sobre preferencias de la hembra por ciertos cantos han sido enfocados principalmente de dos formas: 1) haciendo estudios relacionados directamente con la atracción de la hembra hacia un estímulo particular (p.e. canto con ciertas características) y/o relacionados con la capacidad del macho para que la hembra permanezca con él a lo largo del periodo reproductivo y 2) haciendo estudios que tienen que ver con otras respuestas conductuales de la hembra, principalmente despliegues copulatorios, a ciertos estímulos externos (p.e. un canto con repertorio grande vs un canto con repertorio pequeño). De la misma manera, las investigaciones enfocadas a entender las interacciones entre dos machos que compiten por un territorio o una hembra han tenido dos aproximaciones: 1) estudios que tienen que ver directamente con el éxito o fracaso que confiere un estímulo particular (p.e. canto contra no canto) al competir por un territorio y 2) estudios relacionados con otras repuestas conductuales de

machos a los mismos estímulos (p.e. grabaciones de cantos complejos vs grabaciones de cantos simples). Por otro lado, los estudios de campo enfocados a medir el éxito reproductivo y relacionarlo con algún atributo del canto son la otra cara de la moneda para dar evidencia de los efectos que un tipo de canto puede tener en la reproducción de los individuos.

Es indudable que, aunque los estudios, donde se pone de manifiesto por un lado la atracción de la hembra a ciertos atributos del canto o por otro lado el efecto que puede tener el canto en mantener un territorio libre de intrusos, dan evidencia directa de selección inter e intrasexual respectivamente, las investigaciones que abordan la selección sexual de manera menos directa, como son los estudios que se enfocan a analizar las respuestas conductuales (p.e. despliegues copulatorios en el caso de las hembras y llamados en el caso de los machos) a cantos, son también de gran importancia para entender la evolución y función del canto en las aves.

En general, se piensa que los cantos complejos podrían haber evolucionado principalmente a través de selección intersexual, mientras que cantos más simples y estereotipados podrían ser resultado de selección intrasexual. Este modelo, expuesto por Catchpole (1982), es apoyado con los estudios de Neubauer (1999) con pinzones (*Taeniopygia guttata*), quien mostró que las preferencias de las hembras podrían haber resultado en la evolución de vocalizaciones complejas (i.e. cantos) a partir de otras más sencillas (i.e. llamados).

Sin duda queda mucho por descubrir, y hay otros fenómenos relacionados con el canto que son de gran interés y que, aunque han sido estudiados, nuestra comprensión sobre ellos es menor. Por ejemplo: ¿por qué en algunas especies (sobre todo tropicales) tanto la hembra como el macho cantan en duetos? Si esto es una manera conjunta para proteger el territorio ¿por qué no ha evolucionado esta conducta también en más especies?, ¿por qué en otras especies ocurren coros entre varios individuos?

Por último, la mayoría de los estudios presentados aquí se han enfocado al estudio del canto en aves oscinas (aves “canoras”). Esto es un reflejo de que una gran atención se ha puesto al estudio de estas aves. En cambio, el grupo “hermano” de las oscinas, las suboscinas, ha sido mucho menos estudiado. Aunque no es concluyente, la mayoría de los estudios apuntan a que, a diferencia de las aves oscinas, el desarrollo del canto en las suboscinas no parece estar tan relacionado con el aprendizaje. Esto puede tener implicaciones sobre qué tanta variación podemos encontrar en la producción del canto en estos dos grupos y en cómo utilizan su(s) canto(s) para transmitir diferentes mensajes a

otros individuos. La presente tesis es un esfuerzo por dar más luz e información sobre la variación y uso del canto en una especie de ave suboscina: el cardenalito (*Pyrocephalus rubinus*).

## REFERENCIAS

- Adhikerana A.S. y Slater P.J.B.. 1993. Singing interactions in coal tits, *Parus ater*: an experimental approach. *Anim. Behav.* **6** , 1205-1211.
- Alatalo R.V., Glynn C. y Lundberg A. 1990. Singing rate and female attraction in the pied flycatcher: an experiment. *Anim. Behav.* **9** , 601-603.
- Baptista L.F. 1975. En: Catchpole C.K. y Slater P.J.B. 1995. Bird song. Biological themes and variations. Cambridge University Press.
- Beecher M.D., Stoddard P.K., Campbell S.E. y Horning C.L. 1996. Repertoire matching between neighbouring song sparrows. *Anim. Behav.* **5** , 917-923.
- Buchanan K.L., Catchpole C.K., Lewis J.W. y Lodge A. 1999. Song as an indicator of parasitism in the sedge warbler. *Anim. Behav.* **5** , 307-314.
- Catchpole C.K. 1980. Sexual selection and the evolution of complex songs among European warblers of the genus *Acrocephalus*. *Behaviour* **7** , 149-166.
- Catchpole C.K. 1982. The evolution of bird sounds in relation to mating and spacing behavior. En: Acoustic Communication in Birds (V.1) (Kroodsma D.E. y Miller E.H. Eds.). Academic Press.
- Catchpole C.K. y Slater P.J.B. 1995. Bird song. Biological themes and variations. Cambridge University Press.
- Catchpole C.K., Dittami J., Leisler B. 1984. Differential responses to male song repertoires in female songbirds implanted with oestradiol. *Nature*. **3** , 563-564.
- Doutrelant C., Leitão A., Otter K. y Lambrechts M.M. 2000. Effect of blue tit song syntax on great tit territorial responsiveness- an experimental test of the character shift hypothesis. *Behav.Ecol.Sociobiol.* **8** , 119-124.
- Drăgănoiu T.I., Nagle L. y Kreutzer M. 2002. Directional female preference for an exaggerated male trait in canary (*Serinus canaria*) song. *Proc.R.Soc.Lond. B.* **269** , 2525-2531.
- Eriksson D. y Wallin L. 1986. Male bird song attracts females – a field experiment. *Behav. Ecol. Sociobiol.* **9** , 297-299.
- Gibson R.M. 1989. Field playback of male display attracts females in a lek breeding sage grouse. *Behav. Ecol. Sociobiol.* **24** , 439-443.
- Gibson R.M. y Bradbury J.W. 1985. Sexual selection in lekking sage grouse: Phenotypic correlates of male mating success. *Behav. Ecol. Sociobiol.* **8** , 117-123.
- Gil D. 1997. Increased response of the short-toed treecreeper *Certhia brachydactyla* in sympatry to the playback of the song of the common treecreeper *C. familiaris*. *Ethology* **9** , 632-641.
- Gil D. y Slater P.J.B. 2000. Multiple song repertoire characteristics in the willow warbler (*Phylloscopus trochilus*): correlations with female choice and offspring viability. *Behav. Ecol. Sociobiol.* **4** , 319-326.
- Göransson G., Högstedt G., Karlsson J., Källander H. y Ulfstrand S. 1974. En: Catchpole C.K. y Slater P.J.B. 1995. Bird song. Biological themes and variations. Cambridge University Press.
- Gottlander K. 1987. Variation in the song rate of the male pied flycatcher (*Ficedula hypoleuca*): causes and consequences. *Anim. Behav.* **5** , 1037-1043.
- Groschupf K. y Mills G.S. 1982. Singing behavior of the five-striped sparrow. *Condor.* **8** , 226-236.
- Hasselquist D., Bensch S. y Schantz von T. 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature.* **8** , 229-232.
- Hiebert S.M., Stoddard P.K. y Arcese Peter. 1989. Repertoire size, territory acquisition and reproductive success in the song sparrow. *Anim. Behav.* **3** , 266-273.
- Hoit-Leiner M., Nechtelberger H. y Hoi H. 1995. Song rate as a signal for nest site quality in blackcaps (*Sylvia atricapilla*). *Behav. Ecol. Sociobiol.* **3** , 399-405.
- Ikebuchi M., Futamatsu M. y Okanoya K. 2003. Sex differences in song perception in Bengalese finches measured by the cardiac response. *Anim.Behav.* **6** , 123-130.

- Krebs J.R., Ashcroft R. y Webber M.I. 1978. Song repertoires and territory defence on the great tit. *Nature*. **271** , 539-542.
- Kroodsma D.E. y Parker L.D. 1977. Vocal virtuosity in the brown thrasher. *Auk*. **9** , 783-785.
- Leitão A. y Riebel K. 2003. Are good ornaments bad armaments? Male chaffinch perception of songs with varying flourish length. *Anim.Behav.* **6** , 161-167.
- Martin-Vivaldi M., Palomino J.J., Soler M. y Martínez J.G. 1999. Song strophe-length and reproductive success in a non-passerine bird, the Hoopoe *Upupa epops*. *Ibis*. **4** , 670-679.
- McDonald M.V. 1989. Function of song in Scott's seaside sparrow, *Ammodramus matirimus peninsulae*. *Anim. Behav.* **8** , 468-485.
- Mennill D.J., Ratcliffe L.M. y Boag P.T. 2002. Female eavesdropping on male song contests in songbirds. *Science*. Vol. 296, 873.
- Mountjoy D.J. y Lemon R.E. 1996. Female choice for complex song in the European starling: a field experiment. *Behav. Ecol. Sociobiol.* **8** , 65-71.
- Neubauer R. 1999. Super-normal length song preferences of female zebra finches (*Taeniopygia guttata*) and the theory of the evolution of bird song. *Evol. Ecol.* **3** , 365-380.
- Peake T.M., Terry A.M.R., McGregor P.K. y Dabelsteen T. 2002. Do great tits assess rivals by combining direct experience with information gathered by eavesdropping? *Proc. R. Soc. Lond. B.* **20** , 1925-1929.
- Peek F.W. 1972. An experimental study of the territorial function of vocal and visual display in the male red-winged blackbird (*Agelaius phoeniceus*). *Anim.Behav.* **20**, 112-118.
- Radesäter T., Kakobsson S., Andbjørn N., Bylin A. y Nyström K. 1987. Song rate and pair formation in the willow warbler, *Phylloscopus trochilus*. *Anim.Behav.* **5** , 1645-1651.
- Searcy A. W. y Andersson M. 1986. Sexual selection and the evolution of song. *Ann. Rev. Ecol. Syst.* **1** , 507-533.
- Searcy W.A., Searcy M.H., Marler P. 1982. The response of swamp sparrows to acoustically distinct song types. *Behaviour*. **8** , 70-83.
- Slabbekoorn H. y ten Cate C. 1998. Perceptual tuning to frequency characteristics of territorial signals in collared doves. *Anim. Behav.* **5** , 847-857.
- Smith D.G. 1979. Male singing ability and territory integrity in red-winged black birds (*Agelaius phoeniceus*). *Behaviour*. 68, 193-206.
- Tobias J. A. y Seddon N. 2002. Female begging in European robins: do neighbors eavesdrop for extrapair copulations? *Behav. Ecol.* **5**, 637-642.
- Wasserman F.E. y Cigliano J.A. 1991. Song output and stimulation of the female in white-throated sparrows. *Behav. Ecol. Sociobiol.* **29**, 55-59.
- West M.J., King A.P., Eastzer D.H. 1981a. The cowbird: reflections on development from an unlikely source. *Am. Sci.* **6** , 56-66.
- West M.J., King A.P., Eastzer D.H. 1981b. Validating the female bioassay of cowbird song: Relating differences in song potency to mating success. *Anim. Behav.* **29**, 490-501.
- Wiley R.H., Hatchwell B.J. y Davies N.B. 1991. Recognition of individual males' songs by female dunlocks: a mechanism increasing the number of copulatory partner and reproductive success. *Ethology*. **8** , 145-153.

**Variation in the song of  
a sub-oscine, the  
vermillion flycatcher**

# Variation in the song of a sub-oscine, the vermilion flycatcher

Alejandro Ariel Ríos Chelén<sup>1,2</sup>, Constantino Macías García<sup>1</sup> &  
Katharina Riebel<sup>3</sup>)

(<sup>1</sup> Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México. A.P. 70-275, C.P. 04510, México, D.F., México; <sup>3</sup> Institute of Biology, Behavioural Biology Group, Leiden University, P.O. Box 9516, 2300 RA Leiden, The Netherlands)

(Accepted: 13 July 2005)

---

## Summary

Most studies on song variation have focused on oscine birds, whereas sub-oscine singing has seldom been described in detail, with variation in song structure and performance rarely quantified. Yet this information is required to formulate hypotheses regarding possible coding of individuality, motivational or breeding status, and also for a more informed comparison of oscine versus sub-oscine song. To this end we recorded songs of 12 territorial males of a Central Mexican population of the vermilion flycatchers (*Pyrocephalus rubinus*) throughout the breeding season. We found that: (1) although all males sang only one general form of song, both song structure and performance showed substantial seasonal and inter-individual variation; (2) this variation was most pronounced in the number of introductory elements; (3) after the onset of nest construction, both the number of introductory elements and the song rate changed. We discuss some possible functions of this variation.

*Keywords:* vermilion flycatcher, sub-oscine, song variation, bird song, Tyranidae, individual variation.

## Introduction

Most studies on the function of song have been conducted on oscine songbirds in temperate regions of the world (Catchpole & Slater, 1995); little is

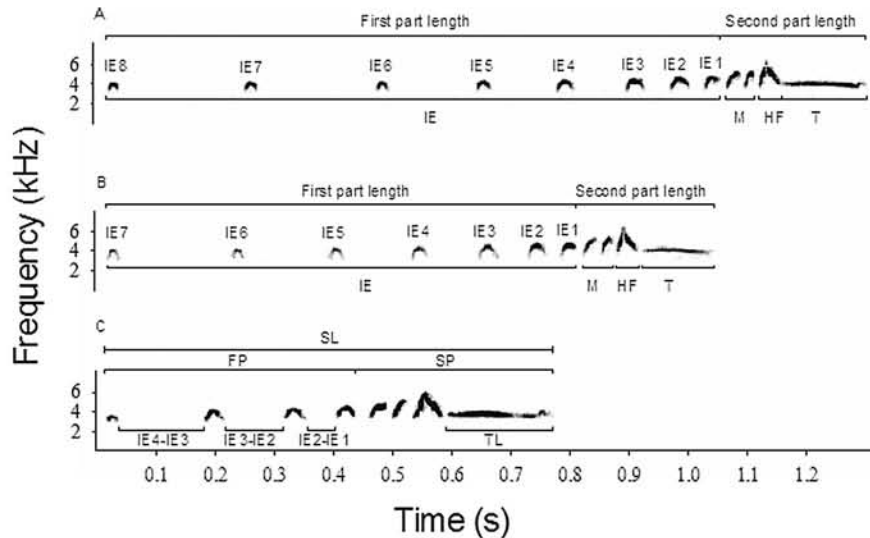
---

<sup>2</sup>) Corresponding author's e-mail address: aarios@miranda.ecologia.unam.mx

known about form and function of vocalizations of sub-oscines. Much of the variation between males in oscine songbirds is thought to result from the fact that songs are learned during ontogeny (Mundinger, 1982; Slater, 1989). Although the evidence is still inconclusive, song in sub-oscines does not seem to be transmitted through social learning processes to the same degree. This assumption is based on several laboratory studies, documenting that sub-oscine birds have developed species-specific songs without exposure to an adult song model (Kroodsma, 1984; Kroodsma & Konishi, 1991). However, sub-oscine birds are often prolific singers and songs seem to play an equally important role in male-female association and in the territorial context as in songbirds (Smith, 1988; Smith & Smith, 1992, 1996a, 1996b) raising the question of how differences in male quality might be reflected in individually specific song characteristics (Martín-Vivaldi et al., 1999; Catchpole, 2000; ten Cate et al., 2002; Forstmeier et al., 2002) and whether and which song parameters might be affected by differences in male condition (Rehsteiner et al., 1998), motivation (Hill & Lein, 1987) or breeding status (Catchpole, 1983). Within or between male variation in song delivery can come about in different ways, such as through the production of a repertoire of different song types, or by variation in frequency or in structure of a single song type. Most studies on the function of song have been concerned with the first type of variation, whereas the importance of the second kind has received less attention (Searcy & Nowicki, 1999). One family of sub-oscine birds in which there is intraspecific variation in the production of song is the Tyrannidae, to which our study species, the vermilion flycatcher (*Pyrocephalus rubinus*) belongs. There are examples of tyrannid species that have only one type of song like the alder flycatcher, *Empidonax alnorum* (Kroodsma 1984) while others have song type repertoires (the willow flycatcher, *Empidonax traillii*, Kroodsma, 1984; the eastern phoebe, *Sayornis phoebe*, Kroodsma & Konishi, 1991; the eastern kingbird, *T. tyrannus*, Smith & Smith, 1992).

In oscine songbirds song repertoires are used in the context of male-male interactions, for instance to deter other males from intruding in the singer's territory (Krebs et al., 1978) or in male-female interactions, for instance when females may seek extra pair copulations (Hasselquist et al., 1996). In species that sing only one type of song, however, individuals cannot be distinguished by song repertoires, and variation in song delivery such as song rate or song length (i.e., adding elements) may be used as clues to individual's identity, quality or motivation to mate or to defend a territory





**Figure 1.** Spectrograms of *Pyrocephalus rubinus* song. Spectrograms A and B are from the same individual. IE = introductory elements. M = middle elements, HF = high frequency element, T = terminal element. Spectrogram C illustrates the temporal measures: song length (SL), first part length (FPL), second part length (SPL). The duration of every element was also measured as illustrated for the terminal element length (TL). The silent interval between all introductory elements (here the intervals IE4-IE3, IE3-IE2, and IE2-IE1) was measured, then the difference in pause duration within every set of three consecutive introductory elements was obtained (e.g., the difference between IE6-IE5 and IE5-IE4, between IE5-IE4 and IE4-IE3 and so on until the first introductory element). These differences were averaged (= average difference in pauses ADP).

(see Wasserman & Cigliano 1991). While some sub-oscine birds seem unable to use song variation to discriminate between distinct songs (Bard et al., 2002), other sub-oscines (e.g., alder flycatcher, *Empidonax alnorum*) may use subtle changes in the frequency or time domain of a single song type to discriminate between neighbour and stranger songs (Lovel & Lein, 2003).

We conducted a study to describe vermilion flycatcher song during the breeding season at around sunrise, when song activity is highest. Territorial male vermilion flycatchers emit a complex vocalization while perching, which was called Regularly Repeated Vocalization (RRV) when first described spectrographically (Smith, 1967). We refer to this Regularly Repeated Vocalization simply as song (Figure 1) because both form and usage seem equivalent to what would be called song in other birds: it is more complex than other vocalizations of vermilion flycatchers (Smith, 1967, 1970;

AARCH, pers. obs.) and males show an increased rate of singing during the breeding season, which is evident before sunrise (AARCH, pers. obs.). As the only other previous recording-based description of vermilion flycatcher song (Smith, 1967) used only three individuals, and lacked fine measures of temporal and frequency parameters, the aims of this study were to arrive to a more detailed description of the temporal and frequency organization of vermilion flycatcher song, to assess the variation in structure and performance within and between males, and to test whether any of the measured song parameters would change between different contexts within the breeding season.

## Methods

### *Study area and subjects*

This study was conducted in the forest of San Diego Metepec (19°17.97N, 98°14.60W), Tlaxcala, Mexico, from February to July 2001. This is a suburban forested area of mixed vegetation, mostly *Pinus* spp. and *Eucalyptus* spp., which is interspersed with clearings and grassland. It is a relatively open area with rather good visibility. The forest is adjacent to the campus of Tlaxcala State University (Universidad Autónoma de Tlaxcala, UAT) and is regularly visited by people walking and exercising.

Most birds at our site appear to be migrants, and during the four years (2000-2003) that we have been studying this population, they have settled in their territories from mid February onwards, with the last male settling as late as mid June. There are two males that have occupied the same territories for the four years, where they have been spotted through the year, suggesting that they may be residents. In spring 2001 we counted 18 territories and conducted focal samples for 16 males; 12 males were observed intensively and their songs were recorded regularly. Of these 12 males, ten could be identified by individual colour band combinations, and the other two by their song post choice. Each banded male typically used the same song perches suggesting that the method for identifying the unbanded males was valid. A male was considered to have settled when observed defending his territory for at least three consecutive days. This involved interacting with other birds, calling, and sometimes chasing other males and females. Once males were settled, their territories were mapped by performing focal observations

of each male and registering their perching behaviour. From these observations it became apparent that males had no 'singing only' or 'feeding only' perches.

The number of neighbours was also assessed. We defined as a neighbour a male that occupied an area that was at least in part adjacent to the territory of the focal male, as determined during the focal observations. During the reproductive season, borders sometimes changed: some males lost parts of their territory when other males arrived, while a few expanded their territories when neighbours left. The first female to start nest building commenced in early April and the first egg was laid around mid April.

#### *Data collection*

We recorded the song of every male at two different dates, early and late in the breeding season. This means that we obtained recordings before and after the onset of nest construction. We could not assess exact pairing dates as most males were seen associated with females as soon as they had settled in a territory. Song was recorded from around 0530 to 0630 h with a Sennheiser ME66 microphone and a Marantz PMD221 cassette recorder. We recorded songs from the beginning of the singing activity (when it was still dark), through the period when the sun was rising and until the male stopped singing. For each period, the best songs (those with least background noise) were digitized for detailed analysis (see sample below) on a PC computer either using a Yamaha® Sound card DS-XG PCI audio (WDM) or a Creative Audio® PCI (ES 1371, ES 1373 [WDM]) sound card. These A/D cards provided anti-aliasing. Songs were high-pass filtered with a cut-off frequency of 2.8 kHz, and sampled at a rate of 22050 Hz using the Avisoft® SASLabPro software. In order to have comparable amplitude levels in all recordings, we took care to keep a similar distance (5-15 meters) to the singing male when recording.

We also assessed song rate before and after nest building took place, by counting the number of songs per minute, including only those minutes during the hour long focal observation period at dawn that the bird actually spent singing (range 11-44 min). We could obtain song rates for 16 territorial males (13 of which were colour banded) before the onset of nest construction. A number of females ( $N = 3$ ) did not lay any egg and therefore their male mates were not considered for analysis of song rate in the after-nest-construction period.

### *Song sample*

To avoid biasing our song sample towards males for which we had several recordings and to ensure that our song sample would cover at least two different phases of the breeding season, we selected 10 songs per male (except for one individual from which we had only nine songs) using the following rules: we first selected for each male a recording from a day previous to the onset of nest construction. Second, we selected another recording ideally from the day, or otherwise the closest possible day, beginning after nest building, until the female laid the first egg (mean = 9.5 days to first egg). For each male, five songs from the period before nest-building and five songs from the period after nest-building (a total of 119 songs from 12 males) were selected for further analyses. The five songs for each period were randomly selected from those digitized (total = 465 songs, mean  $\pm$  SD =  $38.7 \pm 15.3$  songs per male) by asking a naïve observer to draw numbers that had been assigned to the digitized songs from a hat.

### *Measuring the song*

Figure 1A-C shows spectrograms of typical vermilion flycatcher songs. All recorded songs were composed of a number of subunits or elements (defined as a continuous trace on a spectrogram). Songs consisted of two structurally different parts. First, all songs started with an accelerating series of 3 to 9 introductory elements (IE): these chevron-shaped frequency modulated whistles constituted the first part of the song. We numbered these introductory elements by counting from the introductory element closest to the beginning of the invariant part of the song (see below) toward the beginning of the song (labelled IE1, IE2, etc., Figure 1A-B). Secondly, introductory elements were invariably followed by a fixed sequence of 4 elements ('the second part'). The first two had a fast upward frequency modulation and were termed middle elements (M), from left to right first and second middle elements respectively. These were always followed by the high frequency element (HF): this frequency modulated element had the highest frequency within a song. Finally, the last element within a song, of lower average frequency, was named the terminal element (T). This was the longest element and could also be frequency modulated, although less strongly so.

The temporal parameters measured in each song are illustrated in Figure 1C. Spectrograms were calculated using the Avisoft SASLabPro software (FFT-size: 128, frequency resolution: 125 Hz, temporal resolution:

4 ms, window: Hamming). Additionally, we counted and assessed the number of introductory elements (NIE); element rate (ER), which is the total number of elements divided by song duration; introductory element rate (IER), being the number of introductory elements divided by the duration of the first part of the song; final element rate (FER), or number of elements in second part of the song (always four) divided by duration of the second part of the song; average element length (AEL); maximum frequencies of the last introductory element (FIE), of the high frequency element (FH), and of the terminal element (FT); and the difference in frequency (DF), calculated by subtracting the maximum frequency of the last introductory element from the maximum frequency of the high frequency element.

Visual inspection suggested that the pauses between introductory elements were variable, decreasing in time so that the song appeared to accelerate. Therefore, pauses between consecutive introductory elements were measured and the average of differences in pause duration (ADP) between adjacent elements was calculated (see Figure 1C). Average of differences in pauses is a measure of ‘acceleration’ within the first part of a song: higher values of ADP mean that the differences in duration among introductory elements are on average greater (more accelerated) than for those with lower values (less accelerated).

All temporal and frequency related measures were conducted by using the on-screen cursor available in the spectrogram functions of the Avisoft SASLabPro software.

### *Statistical analyses*

We obtained 14 different measures for each song (see: Measuring the song) (Table 1). A number of these parameters were highly correlated with each other (Pearson correlation: number of introductory elements and song length:  $r = 0.84$ ,  $p = 0.001$ , and first part length:  $r = 0.89$ ,  $p = 0.000$ ; terminal element length and final element rate:  $r = -0.91$ ,  $p = 0.000$ , average element length:  $r = 0.91$ ,  $p = 0.000$ , and second part length:  $r = 0.93$ ,  $p = 0.000$ ; element rate and introductory element rate:  $r = 0.80$ ,  $p = 0.001$ ; difference in frequency and average of differences in pauses:  $r = -0.82$ ,  $p = 0.001$ ;  $N = 12$  males in all correlations). For subsequent statistical analyses we therefore proceeded with the original variables, but used only the seven variables that were not inter-correlated (after Bonferoni correction

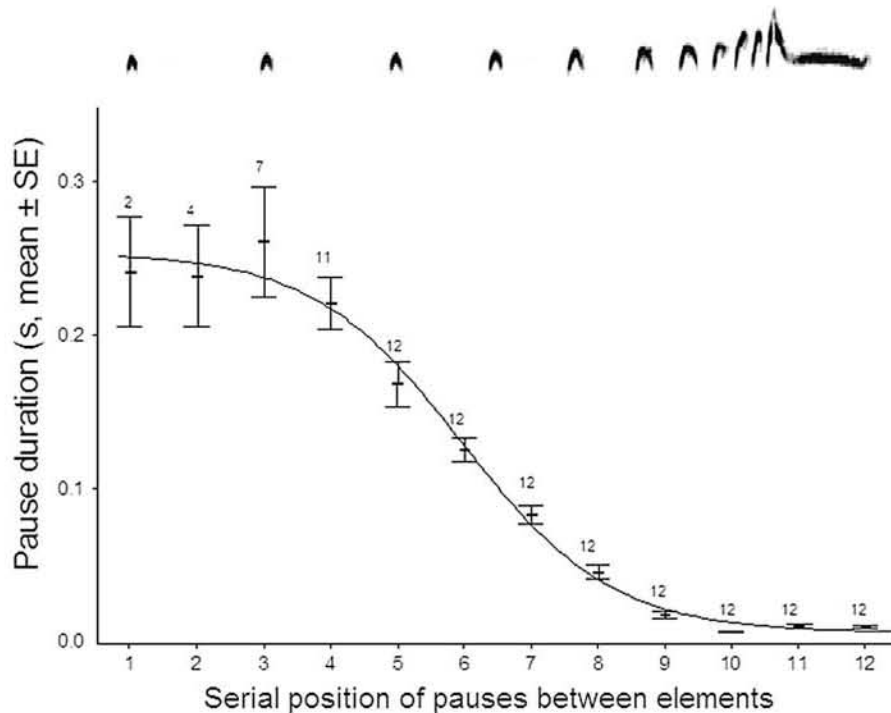
**Table 1.** Mean of means ( $\pm 1$ SD,  $N = 12$  males) for each song parameter. Parameter names are described in the text.

Parameters	Mean $\pm$ SD
Number of introductory elements	5.3 $\pm$ 1
Song length (s)	0.86 $\pm$ 0.14
First part length (s)	0.62 $\pm$ 0.15
Second part length (s)	0.23 $\pm$ 0.02
Terminal element length (s)	0.10 $\pm$ 0.01
Introductory element rate (introductory elements/s)	8.9 $\pm$ 0.8
Final element rate (elements in second part/s)	17.1 $\pm$ 1.8
Element rate (elements/s)	10.4 $\pm$ 1.1
Average of differences in pauses (ms)	40 $\pm$ 10
Average element length (ms)	34 $\pm$ 3
Maximum frequency of last introductory element (Hz)	3730 $\pm$ 150
Maximum frequency of high frequency element (Hz)	5940 $\pm$ 150
Difference in frequency (Hz)	2200 $\pm$ 170
Maximum frequency of terminal element (Hz)	4000 $\pm$ 150

**Table 2.** Repeatability (R) and coefficient of variation (CV) values for non inter-correlated song parameters. To obtain repeatability values for each song attribute using ANOVA, we considered males as groups (see Lessells & Boag, 1987) and pooled the data from before and after nest building periods. The  $F$  ratios and  $p$  values resulting from the ANOVA are shown before each repeatability value.

Parameters	$F$ ratio	$p$ value	R	CV %
Number of introductory elements	9.9	<0.001	0.47	18
Terminal element length (s)	22.8	<0.001	0.68	10
Element rate (elements/s)	4.2	<0.001	0.24	11
Maximum frequency of last introductory element (Hz)	7.5	<0.001	0.40	4
Maximum frequency of high frequency element (Hz)	12.8	<0.001	0.54	2
Difference in frequency (Hz)	7.8	<0.001	0.40	8
Maximum frequency of terminal element (Hz)	12.3	<0.001	0.53	4

$p < 0.002$ ) (Table 2). Parametric tests were applied for normally distributed data, otherwise non-parametric tests were used. Whenever multiple tests on the same data set were performed,  $p$ -values were modified using a sequential Bonferroni correction (Rice, 1989). For any other analysis we used the level of significance of  $p < 0.05$ . We used the SPSS (version 10.0.1) and the Minitab (version 12.21) statistical software packages.

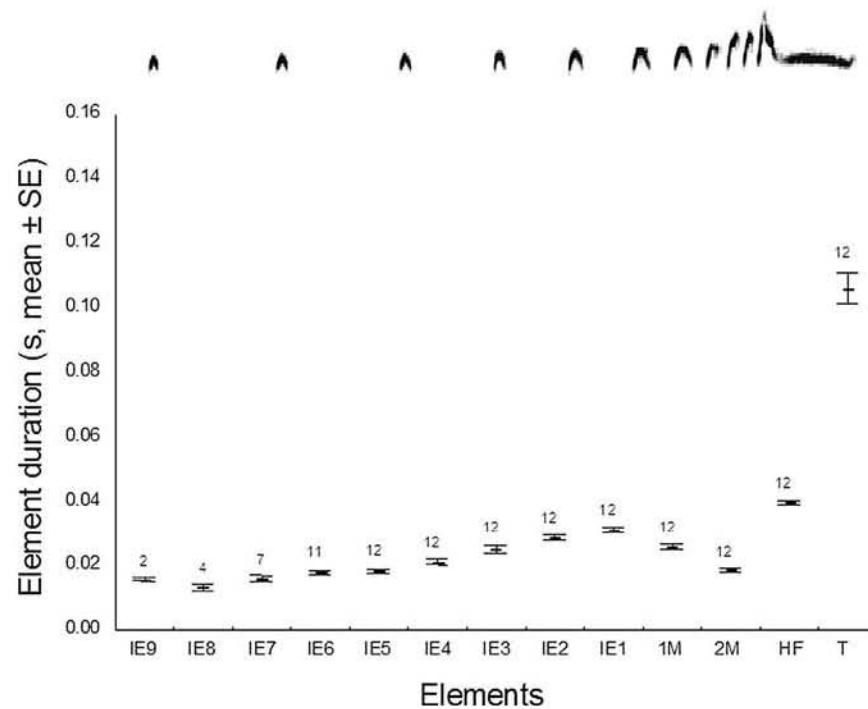


**Figure 2.** Pause duration between elements (grand mean  $\pm$  SE of the mean value obtained from each male) in relation to their position within the song. Sample sizes ( $N$  = number of males) are shown above each data point (not all males sang the maximum number of introductory elements observed). A spectrogram is shown on top of the plot to identify the positions of the pauses within the song.

## Results

### *General description of song*

Songs steadily accelerated as the pause durations between elements decreased towards the end of the song (Figure 2). The equation that fitted the curve in Figure 2 was  $y = 0.0071 + 0.2479/(1 + \text{EXP}(0.894*(x - 5.958)))$  which was significant ( $R^2 = 0.98$ ,  $p < 0.001$ ,  $N = 12$ ). On the other hand, introductory elements continuously increased in duration towards the shorter middle elements. These were followed by the slightly longer high frequency element and the terminal element which had by far the longest duration (Figure 3).



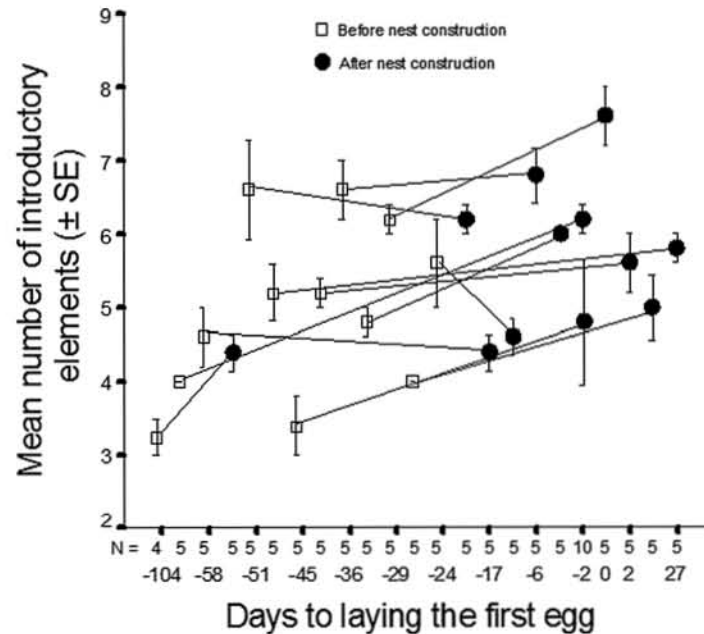
**Figure 3.** Average duration of the different elements within the song (grand mean  $\pm$  SE of the mean value obtained from each male). IE1-IE9 = Introductory elements 1 to 9, 1M and 2M = first and second middle elements respectively, HF = high frequency element, T = terminal element. Sample size ( $N$  = number of males) is indicated above each bar. A spectrogram is depicted above the graphic to aid interpretation.

#### *Variation within and between males*

All song parameters differed significantly between males (Table 2). Comparing the coefficient of variation ( $SD/mean * 100\%$ ) shows that the number of introductory elements (thus also song length as the two were highly correlated, see methods) showed by far the highest variation between males (Table 2).

In order to determine which song parameters were less variable within than among individuals we obtained repeatability values (Lessells & Boag, 1987). A low repeatability value for a particular parameter means this is a relatively variable parameter within an individual, whereas relatively constant parameters have high repeatability values. Repeatabilities were calculated for each song parameter (Table 2). Overall, attributes related to the second





**Figure 4.** Most males (9 of 12) sang songs with more introductory elements after nest building than before nest building. For illustration the two points representing each male (one before and one after nest construction) are linked with a line. Paired  $t$ -test,  $t_{11} = -2.5$ ,  $p < 0.03$ .

part of the song (i.e., terminal element length, maximum frequency of the high frequency element, and maximum frequency of the terminal element) showed higher repeatabilities than those related to the first part of the song, suggesting that these parameters were less variable within individuals.

#### *Variation of song across the breeding season (before/after nest construction)*

Songs produced after nest building had more introductory elements than songs produced before nest building (Figure 4, paired  $t$ -test,  $t_{11} = -2.5$ ,  $p < 0.03$ ). No other song attributes varied significantly between periods (paired  $t$ -test, all  $p > 0.05$ ).

We found a nearly significant increase in the average number of neighbours per male in the period after nest-building when compared with the period before nest-building (paired  $t$ -test,  $t_{11} = -2.1$ ,  $p = 0.054$ ), but the number of neighbours per male was not correlated with the average number

of introductory elements in his song ( $r_s = 0.30$ ,  $p = 0.35$ ,  $N = 12$ ), suggesting that the increase in song length after nest building was not merely a function of a larger number of neighbours present.

Songs with more introductory elements showed a non significant tendency to have shorter terminal elements ( $r = -0.51$ ,  $p = 0.08$ ,  $N = 12$ ).

We found a positive correlation ( $r = 0.62$ ,  $p < 0.02$ ,  $N = 16$ ) between song rate and number of neighbouring males before nest building, but not after nest building ( $r = 0.07$ ,  $p > 0.5$ ,  $N = 13$ ). However, before nest construction song rate also increased with date ( $r = 0.51$ ,  $p < 0.05$ ,  $N = 16$ ), although this trend was not significant after Bonferroni correction (alpha = 0.025). When we controlled for date (performing a partial correlation analysis), song rate was still correlated with number of neighbours ( $r = 0.64$ ,  $p < 0.01$ ,  $N = 16$ ), but when number of neighbours was controlled for, the correlation between song rate and date became non-significant ( $r = 0.55$ ,  $p < 0.05$ ,  $N = 16$ ). Additionally, song rate was higher after nest building than before nest building (paired  $t$ -test,  $t_{12} = -4.9$ ,  $p < 0.001$ )

## Discussion

Although all male vermilion flycatchers in our sample sang the same type of song, there was substantial variation in song delivery between males. Previous studies had not quantified the song in such detail. Smith (1967) had formerly reported no obvious variation between males, and no variation within males, except for slight differences in the number of introductory elements and in the length of the pauses between them. Our analysis allowed us to quantify this assertion, but also showed that there were significant differences among males, as well as a seasonal effect that resulted in within male variation. The results show that vermilion flycatchers use song rate and song length (which increased as a function of the number of introductory elements) as means of achieving variation in song delivery.

Overall, the highest coefficient of variation among males was found in the number of introductory elements, but this parameter also varied intra-individually with the season. Males sang songs with more introductory elements after nest building than before. However, the intra-individual variation was overall smaller than the variation found between males for the same attribute, as shown with the ANOVA's  $p$  value ( $<0.001$  in Table 2). On the

other hand, songs with more introductory elements showed a non significant tendency to have shorter terminal elements. While there was a high inter-individual coefficient of variation for the number of introductory elements, the terminal element length was more repeatable within individuals (Table 2). As the terminal element length also showed a non negligible coefficient of variation (Table 2), we suggest that this attribute could potentially convey information about individual identity.

The observed association between song rate and number of neighbours before nest building was in accordance with the idea that singing is a signal directed to neighbouring territorial males. However, this does not exclude the possibility that these songs also attract and/or stimulate females. It has been suggested that male song rate relates to female mate choice in oscine songbirds, e.g., in the willow warbler (*Phylloscopus trochilus*, Radesäter et al., 1987) and in the blackcap (*Sylvia atricapilla*, Hoi-Leitner et al., 1995). In willow warblers, a negative correlation between song rate and pairing date has been reported (Radesäter et al., 1987). In blackcaps, habitat quality and a measure of female mating preference (the time interval between the onset of male singing and the beginning of egg laying) were correlated with song output and when territory quality and song rate were independently controlled for, only song rate was correlated with female mating preferences (Hoi-Leitner et al., 1995). There is also evidence suggesting that long songs and high song rates may serve to stimulate or attract females, as has been shown in the laboratory for the white-crowned sparrow *Zonotrichia leucophrys* (Wasserman & Cigliano, 1991) and in the field with the pied flycatcher (*Ficedula hypoleuca*, Alatalo et al., 1990). A change in singing after pairing has been reported for *Acrocephalus* warblers (Catchpole, 1983; Catchpole et al., 1986) and this has been interpreted as males and females selecting for different song attributes (see also Searcy & Brenowitz, 1988; Nowicki et al., 1999; Leitão & Riebel, 2003).

These considerations could also account for the observation that song rate after nest building was no longer correlated with the number of neighbours. This suggests that song activity may also be related to female reproductive stage. In our study population nest building starts in early April, and the first eggs are commonly laid in mid April. Nest building and egg laying continue until mid July. Most pairs make more than one reproductive attempt. In this three-month period (April to July), one of our focal pairs had four failed breeding attempts, in all of which the female laid eggs. This implies that,

in a single reproductive year, the female is fertile several times within a period that extends up to several months, and that the period after nest-building (beginning from nest construction), is an extended period in which receptive females can be found. After nest building males not only sang more (increased their song rate), but also sang longer songs. Thus, the observed change in song delivery might be related to female fertility.

The 'fertility announcement' hypothesis (Møller, 1991), which proposes that song is related to mate guarding, such that a paired male announces with his song his female fertile condition and his own willingness to guard her from intruders, has been the subject of controversy (Gil et al., 1999). There are other studies (Pärt, 1991; Ballentine et al., 2003) that have focused on song delivered before or during sunrise only, have shown a correlation between song activity and a period which includes the female fertile period. However, the functions of the dawn chorus remain controversial. For instance, Pärt (1991) found a correlation between singing activity and date, rather than with the female fertile period in the collared flycatcher (*Ficedula albicollis*), and he suggested that the function of song at this time of day is more likely related to maximizing the probability of re-mating in case one's partner disappears. On the other hand, Ballentine et al. (2003) showed that the blue grosbeak (*Guiraca caerulea*) sings more song variants when the female is fertile than during her non-fertile period. Mace (1987) has also given evidence that the dawn chorus in the great tit (*Parus major*) is related to female fertility. She suggested that male dawn song is a display that serves to protect paternity by deterring extra-pair males at dawn, a time when female fertility may be high. Finally, Slagsvold et al. (1994) suggested that dawn singing in the great tit may have multiple functions. Therefore, the possibility that song activity before or during sunrise in the vermilion flycatcher serves to protect paternity should not be discarded. It is also possible that males were singing to attract other females so as to increase their reproductive success by extra-pair mating. These hypotheses are not mutually exclusive, and can be addressed with future studies assessing paternity in relation to song output.

During our study, two males were captured on neighbouring territories before sunrise. This observation, coupled with the increase in song rate as a function of number of neighbours and after the onset of nest building, appears to demonstrate that males can intrude into a neighbour's territory early in the morning, and suggests that territory defence or paternity guarding via

song activity may be particularly important after the onset of nest construction, when the probability of cuckoldry is higher and/or the loss of a territory may be more costly. However, whether territory intrusions are to expand or to take over territories, for foraging or to obtain extra pair copulations has not been tested.

If vermilion flycatcher males use song to protect their territories or paternity, long songs and high song rates might represent more aggressive signals than short songs and low song rates. Male territorial coal tits (*Parus ater*) showed stronger territorial reactions to bouts with an increasing song rate and with long songs than to bouts of a decreasing song rate or short songs (Adhikerana & Slater, 1993). Variation in duration of vocalizations may signal not only level of aggressiveness, but also individual condition, as have been found in doves (*Streptopelia decaocto*), where weight is correlated with an aspect of call structure (ten Cate et al., 2002). It is worth asking whether the parameters that showed high inter-individual variation (number of introductory elements) or that are individual-specific (terminal element length) reliably reflect individual condition or level of aggressiveness in the vermilion flycatcher. Future studies will have to address this by both correlating breeding success with a male's singing activity and by testing different song variants in playback experiments.

#### Acknowledgements

We are grateful to the Centro Tlaxcala de Biología de la Conducta from the Universidad Autónoma de Tlaxcala and in particular to R.A. Lucio and M. Martínez for all their help providing the necessary conditions for the achievement of this study (especially for allowing the use of the laboratory facilities during field work). We thank A. Guillén, L. D'Alba, K. Olea, M. Díaz, P. Escalante and R. Torres for their help with the field work. AARCH is very grateful to all members of the Behavioural Biology group from Leiden University (where the song analysis was carried out), especially C. ten Cate, A. Leitão, S. de Kort, R. Lachlan and G. Beckers. We thank P. Slater and D. Gil for helpful comments on a previous version of the manuscript. W.J. Smith kindly shared opinions and feedback on the topic of this study with us. AARCH was supported by a CONACYT PhD scholarship grant and CMG with a DGAPA (UNAM) research grant.

#### References

- Adhikerana, A.S. & Slater, P.J.B. (1993). Singing interactions in coal tits, *Parus ater*: an experimental approach. — *Anim. Behav.* 46: 1205-1211.

- Alatalo, R.V., Glynn, C. & Lundberg, A. (1990). Singing rate and female attraction in the pied flycatcher: an experiment. — *Anim. Behav.* 39: 601-603.
- Bard, S.C., Hau, M., Wikelski, M. & Wingfield, C. (2002). Vocal distinctiveness and response to conspecific playback in the spotted antbird, a neotropical suboscine. — *Condor* 104: 387-394.
- Ballentine, B., Badyaev, A. & Geoffrey, E.H. (2003). Changes in song complexity correspond to periods of female fertility in blue grosbeaks (*Guiraca caerulea*). — *Ethology* 109: 55-66.
- Catchpole, C.K. (1983). Variation in the song of the great reed warbler *Acrocephalus arundinaceus* in relation to mate attraction and territorial defence. — *Anim. Behav.* 31: 1217-1225.
- Catchpole, C.K. (2000). Sexual selection and the evolution of song and brain structure in *Acrocephalus warblers*. — *Adv. Study Behav.* 29: 45-97.
- Catchpole, C.K., Leisler, B. & Dittami, J. (1986). Sexual differences in the responses of captive great reed warblers (*Acrocephalus arundinaceus*) to variation in song structure and size. — *Ethology* 73: 69-77.
- Catchpole, C.K. & Slater, P.J.B. (1995). Bird song. Biological themes and variations. — Cambridge University Press, Cambridge.
- Forstmeier, W., Kempenaers, B., Meyer, A. & Leisler, B. (2002). A novel song parameter correlates with extra-pair paternity and reflects male longevity. — *Proc. R. Soc. Lond. B* 269: 1479-1485.
- Gil, D., Graves, J.A. & Slater, P.J.B. (1999). Seasonal patterns of singing in the willow warbler: evidence against the fertility announcement hypothesis. — *Anim. Behav.* 58: 995-1000.
- Hasselquist, D., Bensch, S. & von Schantz, T. (1996). Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. — *Nature* 381: 229-232.
- Hill, B.G. & Lein, M.R. (1987). Function of frequency-sifted songs of black-capped chickadees. — *Condor* 89: 914-915.
- Hoi-Leitner, M., Nechtelberger, H. & Hoi, H. (1995). Song rate as a signal for nest site quality in blackcaps (*Sylvia atricapilla*). — *Behav. Ecol. Sociobiol.* 37: 399-405.
- Krebs, J., Ashcroft, R. & Webber, M. (1978). Song repertoires and territory defence in the great tit. — *Nature* 271: 539-542.
- Kroodsma, D.E. (1984). Songs of the alder flycatcher (*Empidonax alnorium*) and the willow flycatcher (*Empidonax traillii*) are innate. — *Auk* 101: 13-23.
- Kroodsma, D.E. & Konishi, M. (1991). A suboscine bird (eastern phoebe, *Sayornis phoebe*) develops normal song without auditory feedback. — *Anim. Behav.* 42: 477-487.
- Leitão, A. & Riebel, K. (2003). Are good ornaments bad armaments? Male chaffinch perception of songs with varying flourish length. — *Anim. Behav.* 66: 161-167.
- Lessells, C.M. & Boag, P.T. (1987). Unrepeatable repeatabilities: a common mistake. — *Auk* 104: 116-120.
- Lovell, S.F. & Lein, M.R. (2003). Neighbor-stranger discrimination by song in a suboscine bird, the alder flycatcher, *Empidonax alnorium*. — *Behav. Ecol.* 15: 799-804.
- Mace, R. (1987). The dawn chorus in the great tit *Parus major* is directly related to female fertility. — *Nature* 330: 745-746.
- Martín-Vivaldi, M., Palomino, J.J., Soler, M. & Martínez, J.G. (1999). Song strophe-length and reproductive success in a non-passerine bird, the hoopoe *Upupa epops*. — *Ibis* 141: 670-679.

- Møller, A.P. (1991). Why mated songbirds sing so much: mate guarding and male announcement of mate fertility status. — *Am. Nat.* 138: 994-1014.
- Mundiger, P.C. (1982). Microgeographic and macrogeographic variation in the acquired vocalizations of birds. — In: Acoustic communications in birds, vol. 2 (Kroodsma, D.E. & Miller, E.H. eds). Academic Press, New York, p. 147-204.
- Nowicki, S., Searcy, W.A., Hughes, M. & Podos, J. (1999). Sexual selection limits evolutionary innovation in birdsong. — *Am. Zool.* 39: 665.
- Pärt T. (1991). Is dawn singing related to paternity insurance? The case of the collared flycatcher. — *Anim. Behav.* 41: 451-456.
- Radesäter, T., Jakobsson, S., Andbjør, N., Bylin, A. & Nystrom, K. (1987). Song rate and pair formation in the willow warbler, *Phylloscopus trochilus*. — *Anim. Behav.* 35: 1645-1651.
- Rehsteiner, U., Geisser, H. & Reyer, H.-U. (1998). Singing and mating success in water pipits: one specific song element makes all the difference. — *Anim. Behav.* 55: 1471-1481.
- Rice, W.R. (1989). Analyzing tables of statistical tests. — *Evolution* 43: 223-225.
- Searcy, W.A. & Brenowitz, E.A. (1988). Sexual differences in species recognition of avian song. — *Nature* 332: 152-154.
- Searcy, W.A. & Nowicki, S. (1999). Function of song variation in song sparrows. — In: The design of animal communication (Hauser, M.D. & Konishi, M. eds). The MIT Press, Cambridge, Massachusetts, p. 577-595.
- Slagsvold, T., Dale, S. & Sætre, G.-P. (1994). Dawn singing in the great tit (*Parus major*): mate attraction, mate guarding, or territorial defence? — *Behaviour* 131: 115-138.
- Slater, P.J.B. (1989). Bird song learning: causes and consequences. — *Ethol. Ecol. Evol.* 1: 19-46.
- Smith, W.J. (1967). Displays of the vermilion flycatcher (*Pyrocephalus rubinus*). — *Condor* 69: 601-605.
- Smith, W.J. (1970). Courtship and territorial displaying in the vermilion flycatcher, *Pyrocephalus rubinus*. — *Condor* 72: 488-491.
- Smith, W.J. (1988). Patterned daytime singing of the eastern wood-pewee, *Contopus virens*. — *Anim. Behav.* 36: 1111-1123.
- Smith, W.J. & Smith, A.M. (1992). Behavioural information provided by two song forms of the eastern kingbird, *T. tyrannus*. — *Behaviour* 120: 90-102.
- Smith, W.J. & Smith, A.M. (1996a). Vocal signalling of the great crested flycatcher, *Myiarchus crinitus* (Aves, Tyrannidae). — *Ethology* 102: 705-723.
- Smith, W.J. & Smith, A.M. (1996b). Playback interactions with great crested Flycatchers, *Myiarchus crinitus* (Aves, Tyrannidae). — *Ethology* 102: 724-735.
- ten Cate, C., Slabbekoorn, H. & Ballintijn, M.R. (2002). Birdsong and male-male competition: causes and consequences of vocal variability in the collared dove (*Streptopelia decaocto*). — *Adv. Study Behav.* 31: 31-75.
- Wasserman, F.E. & Cigliano, J.A. (1991). Song output and stimulation of the female in white-throated sparrows. — *Behav. Ecol. Sociobiol.* 29: 55-59.

## Resumen

La mayoría de los estudios sobre variación del canto se han enfocado a aves oscinas, mientras que la actividad del canto en suboscinos rara vez se ha descrito en forma detallada y casi no

hay cuantificaciones de la variación en estructura del canto y en desempeño. Empero, esta información es necesaria para formular hipótesis relacionadas con una posible codificación de la individualidad, del estado motivacional o reproductivo, y para poder comparar con más información el canto de los oscinos con el canto de los suboscinos. Con este fin grabamos cantos de 12 machos territoriales de papamoscas cardenalito (*Pyrocephalus rubinus*), en una población del centro de México, durante la temporada reproductiva. Encontramos que: (1) aunque todos los machos cantaron una sola forma general de canto, tanto aspectos de la estructura como del desempeño mostraron variación substancial entre machos y a través de la temporada; (2) esta variación fue más pronunciada en el número de elementos introductorios; (3) después del inicio de la construcción del nido, el número de elementos introductorios y la tasa de canto se modificaron. Discutimos algunas posibles funciones de esta variación.

---



**Flight display  
song of the  
vermilion flycatcher**

Wilson Bulletin 116(4):360–362, 2004

## Flight Display Song of the Vermilion Flycatcher

Alejandro A. Ríos-Chelén<sup>1,2</sup> and Constantino Macías-García<sup>1</sup>

**ABSTRACT.**—A number of authors have qualitatively described the songs of Vermilion Flycatchers (*Pyrocephalus rubinus*) given during flight display, but no spectrograms or quantitative analyses are available. We present such a description based on displays of 14 different males. Our analysis confirms the impressions of earlier authors that the flight song is closely similar to that given by perched birds, but also revealed an important difference: most flight display songs also included an extra element known as the *peent* vocalization. The *peent* is also an alarm call, given during male-male and male-female interactions, as well as during foraging bouts. Besides reporting on the common use of *peent* vocalizations by perched Vermilion Flycatchers, we now describe the frequency and use of *peent* vocalizations in flight display songs. Received 4 March 2004, accepted 29 October 2004.

The repertoire of displays by the Vermilion Flycatcher (*Pyrocephalus rubinus*) includes a conspicuous flight display given during the breeding season (March to August, AARC pers. obs.). This display has been described (De Benedictis 1966, Smith 1967) and related to “territorial proclamation” (Smith 1970: 488). Anecdotal observations (AARC pers. obs.), where a male seems to respond with a flight display to a neighbor’s flight display, support the idea that individuals use this behavior in a male-male context. An interesting feature of flight displays is that they are accompanied by vocalizations. These vocalizations are considered to be closely related to the Regularly Repeated Vocalization, which is normally sung from a perch (Smith 1967, 1970). To add to our knowledge on the nature of songs uttered during flight displays, we present spectrograms of these songs ( $n = 14$  males).

Our study was carried out in the Bosque de San Diego Metepec (19° 18' N, 98° 15' W),

Tlaxcala, México, from 6 March to 11 April 2001. Most observations and recordings were conducted from 08:00 to 11:00 or from 16:00 to 18:00, when birds were more active. Most males ( $n = 11$ ) were banded and identified by their color-band combinations, whereas three males were identified by their choice of song post. Color-banded males typically used the same song perches; these perches were never used by other males, suggesting that the method for identifying unbanded males was appropriate. We mapped all territories and documented the number of neighbors for each male (mean =  $2.57 \pm 1.28$  SD, range: 0–4). The territories were mapped by registering which perches were commonly used by males, noting male-male interactions (i.e., calling) that commonly occurred at territorial boundaries, and recording chases between territory owners and intruding males. A neighbor was defined as a male that had at least part of his territory adjacent to that of the focal male.

We recorded songs with a Sennheiser Me66 microphone and a Marantz PMD221 cassette recorder. Each male ( $n = 14$ ) was recorded during one, 30-min period of observation. If the focal male had not performed a flight display within 30 min, we shifted to another focal male. This was done until all 14 males were recorded. All the males were paired, and although there were differences in the date each male was recorded, all 14 were recorded before their mates commenced nest construction.

Our sample size varied considerably between individuals (from 5 to 16 songs recorded per individual); however, we analyzed an equal number of songs per bird. We used either the whole sample (when  $n = 5$  songs) or randomly selected five songs per individual (when  $n > 5$  songs) resulting in a total of 70 songs analyzed. Songs were digitized on a PC computer using a sampling rate of 22,050 Hz. Songs were high-pass filtered with a cut-off frequency of 2.8 kHz. All variables were mea-

<sup>1</sup> Dep. de Ecología Evolutiva, Inst. de Ecología, Univ. Nacional Autónoma de México, AP 70–275, C.P. 04510, México, D.F., México.

<sup>2</sup> Corresponding author; e-mail: aarios@miranda.ecologia.unam.mx

TABLE 1. Song variation among male Vermilion Flycatchers was statistically significant for most parameters, but variation in the number of *peent* vocalizations was only marginally significant ( $n = 14$  males). Data from recordings made at Bosque de San Diego Metepec, Tlaxcala, México, 6 March–11 April 2001.

Parameter	Mean $\pm$ SD	Statistics: ANOVA or Kruskal-Wallis
Song duration (sec)	0.43 $\pm$ 0.11	$H = 40.61$ , $df = 13$ , $P < 0.001$
Minimum frequency (kHz)	3.49 $\pm$ 0.18	$F_{13,56} = 6.58$ , $P < 0.001$
Maximum frequency (kHz)	5.97 $\pm$ 0.21	$F_{13,56} = 7.80$ , $P < 0.001$
Number of total elements	6.55 $\pm$ 0.65	$H = 42.27$ , $df = 13$ , $P < 0.001$
Number of <i>peent</i> vocalizations	0.74 $\pm$ 0.50	$H = 21.87$ , $df = 13$ , $P = 0.057$
Number of common elements	5.81 $\pm$ 0.85	$H = 36.61$ , $df = 13$ , $P < 0.001$

sured on spectrograms calculated with AVISOFT (Specht 2002) software (FFT: 128; frequency resolution: 125 Hz; temporal resolution: 4 ms; window: Hamming). We measured song duration, minimum and maximum frequency, number of total song elements, number of *peents*, and number of common elements (Table 1). Means are presented  $\pm$  SD.

Flight display songs had the same basic structure and shared the same elements as the ones uttered while perched (Fig. 1A; Smith 1967). Elements found both in songs produced during flight displays and in songs uttered while perched are referred to as common elements. Songs had a mean of 6.55 elements  $\pm$  0.65 (range: 5–8,  $n = 70$ ), of which 5.81

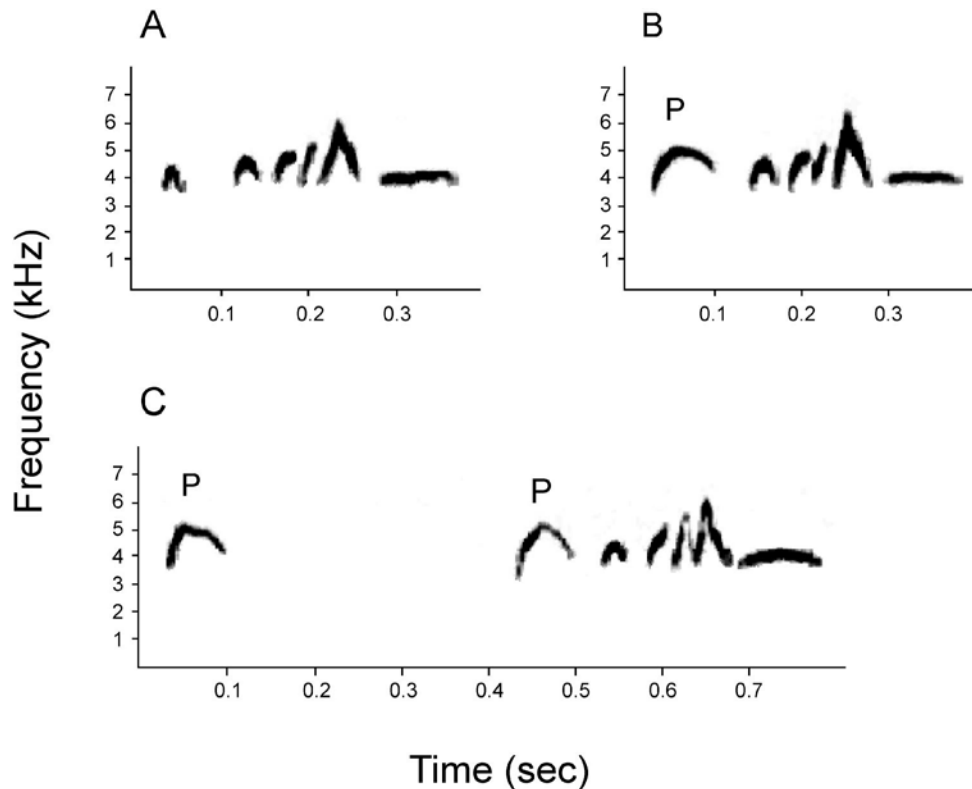


FIG. 1. Songs of Vermilion Flycatchers given during flight displays have a number of common elements (i.e., elements also found in songs given at a perch) and may include the *peent* vocalization. “A” represents a song with common elements. Spectrograms “B” and “C” represent songs that have one or two *peent* vocalizations (P). Recordings were made at Bosque de San Diego Metepec, Tlaxcala, México, 6 March–11 April 2001.

$\pm 0.85$  were common elements (range: 5–7,  $n = 70$ ). Songs had a mean of  $0.74 \pm 0.50$  *peent* vocalizations (range: 0–2,  $n = 70$ ). In our study, 71.3% of the flight display songs had the *peent* vocalization; 68.5% had only one *peent* vocalization, 2.8% had two, and 28.7% lacked the *peent* vocalization. Songs lasted between 0.25 and 0.95 sec (mean =  $0.43 \pm 0.11$ ), the minimum frequency ranged between 3.0 and 3.8 kHz (mean =  $3.49 \pm 0.18$ ), and the maximum frequency ranged between 5.5 and 6.4 kHz (mean =  $5.97 \pm 0.21$ ).

The *peent* vocalization has been previously described by Smith (1967; Fig. 1B, C) and is an alarm call, is given in male-male and male-female interactions, and when foraging (Smith 1967, Wolf and Jones 2000; AARC pers. obs.). As part of another study (AARC, CMG, and K. Riebel unpubl. data), we recorded songs of perched Vermilion Flycatchers given during the dawn chorus, and none of 119 songs included the *peent* vocalization. Thus, the *peent* does not constitute an element in songs of perched birds (AARC pers. obs.; see also Smith 1967).

All males sang at least one song with a *peent* vocalization; most individuals ( $n = 13$ ) gave 0–1 *peents*. We found only one male that sang flight songs with a range of 1–2 *peent* vocalizations. Most males ( $n = 8$ ) had either 5–6 ( $n = 5$  males) or 6–7 ( $n = 3$  males) common elements; four males had 5–7 ( $n = 3$  males) or 6–8 ( $n = 1$  male). All the songs of two males contained five common elements. Songs with a greater number of *peent* vocalizations had fewer common elements. We found a negative correlation between the number of common elements in a song and the number of *peent* vocalizations ( $r = -0.60$ ,  $P = 0.023$ ,  $n = 14$ ), suggesting a trade off be-

tween these two groups of elements. We also found a negative correlation between the number of neighbors and the total number of elements in a song ( $r = -0.63$ ,  $P = 0.016$ ,  $n = 14$ ), suggesting that different numbers of elements in songs may convey different messages to male Vermilion Flycatchers.

Finding a call (the *peent* vocalization) incorporated into a stereotyped song opens the question of whether this represents a variation in repertoire—whereby males modify song structure in different contexts. The relative importance of the *peent* vocalization and the number of elements in flight display songs in male-male and male-female communication must be addressed with playback experiments.

#### ACKNOWLEDGMENTS

We thank R. A. Lucio and M. Martínez for providing the necessary support for field work. D. Gil made helpful comments on a previous version of the manuscript. AARC was supported by a CONACYT Ph.D. scholarship grant and CMG with a Dirección General de Asuntos del Personal Académico (Universidad Nacional Autónoma de México) research grant. We thank three anonymous referees who greatly improved the quality of this manuscript.

#### LITERATURE CITED

- DE BENEDICTIS, P. 1966. The flight song display of two taxa of Vermilion Flycatcher, genus *Pyrocephalus*. *Condor* 68:306–307.
- SMITH, W. J. 1967. Displays of the Vermilion Flycatcher (*Pyrocephalus rubinus*). *Condor* 69:601–605.
- SMITH, W. J. 1970. Courtship and territorial displaying in the Vermilion Flycatcher, *Pyrocephalus rubinus*. *Condor* 72:488–491.
- SPECHT, R. 2002. Avisoft-SASLab Pro: sound analysis and synthesis laboratory user's guide, ver. 4.1. Avisoft Bioacoustics, Berlin, Germany.
- WOLF, B. O. AND S. L. JONES. 2000. Vermilion Flycatcher (*Pyrocephalus rubinus*). *The Birds of North America*, no. 484.

**Effects of song length  
on territorial  
responses during  
song playback  
in a sub-oscine bird**

**Effects of song length on territorial responses during song playback in  
a sub-oscine bird**

Alejandro A. Ríos Chelén<sup>†</sup> & Constantino Macías Garcia

*Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad  
Nacional Autónoma de México. A.P. 70-275, C.P. 04510, México, D.F., México*

<sup>†</sup>Corresponding author e-mail: [aarios@miranda.ecologia.unam.mx](mailto:aarios@miranda.ecologia.unam.mx)

Constantino Macías Garcia e-mail: [maciasg@servidor.unam.mx](mailto:maciasg@servidor.unam.mx)

## Abstract

Vermilion flycatchers (*Pyrocephalus rubinus*) vary their song rate and song length across the breeding period: males sing more and longer songs after nest construction than before. Here we explored the possibility that this variation is meaningful to territorial males. Using a playback approach, we tested several males with different variations in song output (i.e. variations in song length and song rate). We found that males discriminate between long and short songs, and vocalize more when exposed to long songs than to short songs, which suggest that long songs are perceived as more threatening signals than short songs. We did not find evidence of males discriminating between a high and a low song rate. We discuss some possible implications for song function in this sub-oscine species, and compare these findings with other results in oscine species.

## Introduction

In the well studied oscine birds, there is interesting variation on whether species use different song types in different contexts (i.e. intra- and inter-sexual interactions), while others sing the same song type regardless of the context. For instance, great reed warbler's (*Acrocephalus arundinaceus*) long songs are mainly used in the context of female attraction, while short songs are used for territorial defence (Catchpole et al. 1986, Catchpole 2000). Other species may use any of their structurally similar song types in both contexts, but song variation in structurally different parts may code different messages. For example, male and female chaffinches (*Fringilla coelebs*) differ in how they weigh the relative duration of the trill part of a song and the complex final phrase ('flourish') (Leitão and Riebel 2003). On the other hand, how structural variation of song in sub-oscine birds functions in male-male competition and female choice has been little studied. There are examples of sub-oscine species with a song repertoire, e.g. the great crested flycatcher, *Myiarchus crinitus* (Smith and Smith 1996), or with a single song type like the alder flycatcher, *Empidonax alnorum* (Kroodsma 1984) and our study species the vermilion flycatcher, *Pyrocephalus rubinus* (Smith 1967, Ríos Chelén et al. 2005, but see Ríos-Chelén and Macías Garcia 2004).

The great crested flycatcher may use different forms of songs when associating with a female or when interacting with a neighbouring male (Smith and Smith 1996). However, it remains largely unknown how sub-oscine species, particularly those with a single song type, may use their only song type in different intra- and inter-sexual contexts. The evidence so far indicates that a single song type species like the alder



flycatcher can discriminate between neighbours and strangers' songs (Lovell and Lein, 2004).

Here we studied if vermilion flycatchers are able to discriminate between different song variants of their single song type. The vermilion flycatcher's song has already been described (Smith 1967, Ríos Chelén et al. 2005). During the breeding season, males sing mostly before and at dawn, and again at sunset, but singing at other times of the day (from perches or during flight displays) also occurs (De Benedictis 1966, Smith 1967, Archer 1996, Ríos-Chelén and Macías Garcia 2004). Vermilion flycatcher's singing before and at dawn consists of bouts of about 4 songs long (mean  $\pm$  SE =  $4.23 \pm 0.32$  songs/bout, range = 2.0 – 7.1 songs/bout; Ríos Chelén, unpublished data). Natural observations before and at dawn suggest that inter-bout intervals decrease as sunrise time decrease. In other words, vermilion flycatchers apparently sing more constantly (i.e. with shorter inter-bouts intervals) as sunrise time is closer. Ríos Chelén et al. (2005) showed that males increase song length (adding introductory elements) and song rate after the onset of nest construction, and also found a positive correlation between song rate and number of neighbours before the onset of nest construction. The possible function of these changes is unknown. The fact that vermilion flycatchers seem to counter sing with neighbours at dawn (Smith 1967, Ríos Chelén, pers. obser.) suggest that song has a function in male-male communication.

In species like the vermilion flycatcher that lack a repertoire, information regarding quality and motivation of the singer can only be coded in the form of variations within the single song type by varying the number of elements in a song or varying the song rate (Wasserman and Cigliano 1991). If this variation is meaningful to (male) receivers, we predict that territorial male vermilion flycatchers should show

different reactions to playbacks of long and short songs and between a high and a low song rate. Work in oscine birds has shown that different aspects of song might be of different importance at different stages in the breeding season (Mace 1987, Cuthill and Macdonald 1990, Ballentine et al. 2003). We therefore also tested whether responses to song variation changed before and after nest construction took place; the former being a time with high inter-male competition (establishing of territories), the latter a time where males might sing to deter intruders or neighbours to guard paternity and/or to attract extra-pair mates. If the observed change in song delivery (i.e. males singing longer songs and more after nest construction than before, and males singing more when they have more neighbours; Ríos Chelén et al, 2005) is related to female fertility or to territory tenure, one possibility is that males use long songs and/or a high song rate to deter other males from intruding in the singers' territory, whereas to protect paternity (see Møller, 1991) or some resource (e.g. territory). We hypothesize that long songs and a high song rate are used as more threatening signals than short songs and a low song rate. If this is the case, we predict that males should respond more strongly to long songs than to short songs, and more to a high song rate than to a low song rate.

## **Methods**

### **General**

The experiment was carried out in 2003 in the Forest of San Diego Metepec (19° 17.97 N, 98° 14.60 W), in the State of Tlaxcala, Mexico, where we have studied a colour ringed population since 2000. Part of this area is located within the territory of the

Universidad Autónoma de Tlaxcala (UAT). The forest is composed mainly of *Pinus* sp. and *Eucalyptus* sp. with interspaced clearings and grasslands. Most birds in our study population appear to be migrants, leaving the territories around August or September, and returning in January.

The songs used for this experiment were recorded in the same study site where the experiment took place, but 2 yr earlier, in 2001. Songs were recorded at dawn with a Sennheiser ME66 microphone and a Marantz PMD221 cassette recorder, and digitized (number of bits: 16) using a 0 AliAudio Wave 5.10 sound card. Songs were high pass filtered with a cut-off frequency of 2.8 kHz and sampled at a rate of 22050 Hz using the Avisoft® SASLabPro software. We took care that during the experiment no individual was exposed to songs recorded from him or from a male known to have been his neighbour in any of the four years that we have studied this population (2000-2003). All replicates were prepared with different songs, and each individual was exposed to a paired treatment (long vs short songs and high vs low song rate). The high-low rate pairs were made with the same song (i.e. 25 songs/min and 15 songs/min), a different one for each male, and thus any other variable apart of rate was controlled for. The long-short song pairs were prepared with songs from different males, thus we avoided the possibility that males could fail to discriminate as a result of listening songs from the same individual (i.e. songs that shared the same individual identity attributes, Ríos Chelén et al, 2005). Treatments (see Fig. 1) were prepared using the Avisoft® SASLabPro software. Long songs lasted in average 1.21 sec (mean  $\pm$  se =  $1.21 \pm 0.04$ ), and short songs 0.61 sec (mean  $\pm$  se =  $0.61 \pm 0.02$ ).

For the playback experiment we used a Mineroff SME-AFS loudspeaker connected by a 10-m cable to a Sony D-EJ621 CD player. The songs were played at a

sound pressure level of 65-70 db measured at a distance of 8 m with a digital sound level meter (RadioShack, Cat. No. 33-2055). The experiment took place from the 12th of March to the 2nd of July of 2003, and from 0800 to 1330 h. The loudspeaker was placed in a tree judged to be obviously inside the territory of the experimental male, and we took care to locate the loudspeaker in the same place each time we performed a trial with the same male.

A trial was composed of three phases: one control pre playback minute (silence), one playback minute (songs), and a post playback minute (silence). Birds were exposed to two groups of stimuli which we had manipulated to mirror some of the variation we had observed in natural songs (Ríos Chelén et al, 2005): 1) long-short songs: songs had either 3 and 7 or 4 and 8 introductory elements and 2) high–low song rate: songs of equal length, played back at a rate of either 15 or 25 songs per min. These song rates fall well within the natural range (approximately 4 to 30 songs per min, Ríos Chelén, unpublished data). In both cases (i.e. high and low rates) we used the same song when testing a particular individual and this was always a song with 5 introductory elements. Thus, we avoided the potentially confounding effects of number of elements or song length variation in the song rate experiment. Birds were tested with both groups of stimuli (Fig. 1) in two different stages: 1) before nest construction began and 2) after the onset of nest construction.

The series of playback before the onset of nest construction were performed in average 32 days before the first egg was laid (mean  $\pm$  SE =  $32.6 \pm 3.99$ , range = 18-47 days before the first egg was laid). The second set of playbacks, after the onset of nest construction, were performed in average 13 days after the first egg was laid (mean  $\pm$  SE =  $13.2 \pm 3.28$ , range = 0 - 30 days after the first egg was laid), but in average 4 days

before the last egg was laid (mean  $\pm$  SE =  $4.7 \pm 8.32$ , range = from 43 days before to 28 days after the last egg was laid). Vermilion flycatchers can nest several times (i.e. for instance if their eggs are predated) within a single breeding cycle (i.e. up to four or five times). This means that females can be potentially fertile several months in a single reproductive year (i.e. in 2003, at our study population, this was from around April to June). Therefore, we should not discard the possibility that any observed change in response to playback after the onset of nest construction could be related to female fertility.

In order to control for order effects, half of the birds were tested first with variable song length while the other half were tested first with variable song rate. Additionally, for the variable song length experiment, half of the birds were tested with 3 vs 7 introductory elements songs and the other half with 4 vs 8 introductory elements. One day was used to test a particular male with one group of stimuli (e.g. variable song length), in which we left at least 10 min between treatments of short vs long songs, and in the next day the same bird was exposed to the other group of stimuli (e.g. variable song rate) with its corresponding treatments of high and low song rate. All trials were inversed within subjects when repeated in the next breeding period (i.e. after nest construction took place). Since before nest construction half of the birds were tested with one stimulus first (e.g. long songs), and after nest construction the order was inversed for the same males, we controlled for a within subjects order effect between different moments of the season. The number of days between the first set (before nest construction) and the second set (after nest construction) of playbacks differed between individuals and ranged from 21 to 56 d.

We have observed that territorial vermilion flycatcher males involved in natural encounters with intruders display a variable group of behaviours, from which the most common and evident are calling and flying. A male owning a territory will respond approaching and calling to an intruder placed in the frontier of the owner's territory. If the intruder goes further inside the owner's territory, the latter will continue to call and will start chasing the intruder until he is outside of the territory. Although vermilion flycatchers sing mostly at dawn, territorial males sporadically sing during the day and we do not discard the possibility that this might be another behaviour related to potential intruders or neighbours. Therefore, during the playback experiment we registered the following behaviours: number of calls, songs and flights from perch to perch.

### **Analyses**

For several reasons, the number of birds for analysis varies with the treatments, with the behaviour analysed, or with the stage (before/after nest construction began). Our sample size ranged between 6 and 12 analysed males (see below).

To determine whether or not song playback elicited a response by males, we analysed the frequency of response during the pre-, play- and post-playback minutes with a Friedman test. Following Langemann et al. (2000), in those cases in which there was a significant response, we performed the Wilcoxon-Wilcox post hoc test for related samples (Lothar 1997) to reveal those groups (i.e. pre, play and post playback minute) that differed from each other.

In exploratory analyses it became apparent that birds showed a (non-significant) trend to respond with songs occasionally. In other words, we found a non-significant tendency ( $P = 0.039$ ) of birds to respond with songs to playback of long songs before nest construction (Table 1). Additionally, birds' responses to playbacks were statistically very similar when vocalizations (i.e. calls plus songs) were taken as the response variable and when only calls were considered for analyses (Table 1). In other words, all response instances (i.e. vocalizations or calls) during post playback did not statistically differ to the playback min, but did so when compared to the pre playback min. In all cases there was a positive and strong correlation between the call response and the vocalization response during the playback minute (before nest construction: long songs,  $r = 0.99$ ,  $n = 12$ ; short songs,  $r = 0.99$ ,  $n = 12$ ; high rate,  $r = 1$ ,  $n = 10$ ; low rate,  $r = 0.91$ ,  $n = 10$ ;  $P < 0.001$  in all instances. After nest construction: long songs,  $r = 0.99$ ,  $P < 0.001$ ,  $n = 11$ ; short songs,  $r = 1$ ,  $P < 0.01$ ,  $n = 11$ ; high rate,  $r = 1$ ,  $P < 0.001$ ,  $n = 10$ ; low rate,  $r = 0.95$ ,  $P < 0.001$ ,  $n = 9$ ). Therefore, in an effort to reduce the number of response variables and, at the same time, to maintain all vocalizing response as a probably meaningful biological variable, we pooled the variables "calls" and "songs" into a single variable (i.e. "vocalizations") for further analyses. Since birds did not respond significantly with flights to any of the treatments (Table 1), flights were not included in further analyses.

During the course of the experiment, when playback elicited a response, it was evident that males continued responding during the post-playback min of silence, and it was subsequently demonstrated that the frequency of responses during the post playback minute did not differ statistically from that during the playback min (Table 1). Therefore, to further analyse the data we pooled together the playback and post-

playback minutes and compared them with the pre-playback minute. This was done by subtracting the frequency value of “response” during the pre-playback minute from the summed response during the play- and post-playback minutes. In this way we obtained a difference in response to compare between the long and short songs treatments, and between the high and low song rate treatments. This procedure can also give more accurate information about the males’ responses since birds do not normally stop responding all at once when the playback minute has ended. To control for time, in all subsequent analyses we used rates of response (response/min).

As the experiment was designed to compare the response of each individual with itself, we used Two Way Repeated Measures ANOVAs to compare the vocalizations response of birds to different treatments (i.e. long vs short songs, and high vs low song rate) in different periods of the breeding cycle (i.e. before and after nest construction). When a significant difference was detected we performed a Tukey Post hoc test to reveal those groups that differed from each other. Data were previously shown to conform to a normal distribution. We used the SPSS (version 10.0.1.), and Sigma Stat (version 2.0) statistical software. Whenever multiple tests were carried out with the same data set we performed a Sequential Bonferroni correction (Rice 1989).

## **Results**

### **Response to playbacks**

Before nest construction almost all treatments prompted calling and overall vocalization, but the tendency to respond was not significant in the low song rate treatment after Bonferroni (Table 1). Exposure to playback did not lead to changes in



song or flight frequencies in this period, other than non-significant tendencies to respond with flights when long songs and a high rate were played (after Bonferroni,  $\alpha < 0.021$ ), and with songs when long songs were played (after Bonferroni,  $\alpha < 0.039$ ) (Table 1).

After nest construction began, only the long songs treatment prompted a significant increase in calls and vocalizations, whereas responses to all other treatments were non-significant ( $\alpha < 0.018, 0.021, 0.023, \text{ and } 0.039$  for calls, flights, vocalizations, and songs respectively, after Bonferroni: Table 1).

### **Effect of playback treatment**

#### Long vs short songs.

There was a significant effect of breeding period on the vocalization response of birds (Two Way Repeated Measures ANOVA:  $F_{7,1} = 14.30, P = 0.007$ ). Males showed a non significant tendency to respond differentially to long and short songs when these responses were compared across periods (Two Way Repeated Measures ANOVA:  $F_{7,1} = 4.66, P = 0.068$ ). In other words, males did not respond differently between long songs, during the first and second period, and short songs, during the first and second period. However, birds responded more to long songs than to shorter songs before the onset of nest construction, but not after nest construction (Post hoc comparisons: Table 2; Fig. 2). If this apparent difference in response was a result of males habituating to playback, we should expect a lack of response to long songs after nest construction. This was not the case, as males strongly responded to long songs after nest construction (Table 1).

### High vs low song rate.

There was no effect of breeding period on vocalization response when a high and a low song rates were played (Two Way Repeated Measures ANOVA:  $F_{5,1} = 0.30$ ,  $P = 0.606$ ). Males did not respond differentially to a high and a low song rate (Two Way Repeated Measures ANOVA:  $F_{5,1} = 0.004$ ,  $P = 0.949$ ). This negative result could be consequence of a low sample size ( $n = 6$ ), resulted from excluding from the analysis those individuals that were not represented both before and after nest construction. To overcome this possibility, we performed Paired t-tests for each breeding period separately, thus using a bigger sample size. We did not detect a differential response to the song rate treatments on vocalization response either before (Paired t test:  $t = 0.34$ ,  $df = 9$ ,  $P = 0.73$ ) or after nest construction, (Paired t test:  $t = -0.44$ ,  $df = 9$ ,  $P = 0.67$ ).

## Discussion

The most obvious responses of males to our playbacks were vocalizations, mostly calls. That males responded to playback shows they may perceive con-specific songs as threatening signals and that song is used in this species as means of intra-sexual interactions.

Males not only responded to playback, they also responded more to long than to short songs before the onset of nest construction. Since in the long-short songs experiment birds were exposed to treatments with differing song output (long songs represent a higher song output than short songs), the observed discrimination between long and short songs could be due to birds being exposed to treatments of differing song output, and perhaps not to treatments differing in song length. However, our negative

results (i.e. an apparent lack of discrimination between a high and a low song rate) in the song rate experiment suggest that, at least for the measured behaviour (i.e. vocalizations), song output did not result in the observed discrimination between long and short songs. Song length seems to be the cause of the observed discrimination. Our results support our prediction (i.e. that males should respond more strongly to long than to short songs), and show that males discriminate between songs which differ in an attribute that has been shown to vary in nature in relation to the breeding cycle (i.e. song length, Ríos Chelén et al. 2005). Our results can be taken as evidence that song length conveys information relevant for male-male communication in the vermilion flycatcher.

Birds' perception to songs seemed to change with the breeding cycle, as males discriminated between long and short songs before nest construction, but apparently not after nest construction. This may be related to the breeding period (i.e. before or after nest construction), and not to habituation to playback, as birds still responded to playback of long songs after the onset of nest construction. The overall response before nest construction was stronger than after nest construction, suggesting that birds vocalize more in the face of an intrusion before the onset of nest construction, when territories are being settled. However, there is still the possibility that a differential response after nest construction might have been evident had we looked for other behavioural variables (e.g. tail flicking; or even cardiac response, Ikebuchi et al. 2003).

Observations of natural male-male interactions show that when an intruder invades an occupied territory and remains close to the territory border, the owner approaches and immediately starts calling (pers. obs, AARCh). These observations combined with the present results suggest that males perceive long songs as more threatening signals than short songs. McGregor and Horn (1992) suggested that in the

great tit (*Parus major*), strophe length can contain information about the strength of response a male is able to produce in male-male interactions. In line with this, Balsby and Dabelsteen (2001) demonstrated that the whitethroat (*Sylvia communis*) mounts a greater response to a small repertoire that has been elongated in the time domain than to a small repertoire of “normal” length, suggesting that song length rather than repertoire size indicates the degree of arousal of an individual. Therefore, this apparent property of song length of conferring information on the degree of threat might be relatively common to both oscines and sub-oscine birds.

The opposite interpretation is also possible: that longer songs betray less powerful rivals, and being less threatening signals they evoke higher responses by the owner of the territory, as have been suggested for the great reed warbler (*Acrocephalus arundinaceus*: Catchpole et al. 1986). However, based on the observations of natural encounters described above and our results, we are rather inclined to interpret our results as longer songs being more threatening signals. Hoelzel (1986) found that the robin (*Erithacus rubecula*) uses shorter songs in an aggressive intra-sexual context, whilst longer songs are used in response to female songs. We were not able to study detailed female responses to playback in the vermilion flycatcher, although sporadic observations suggest that females also flight and call in response to playback (Ríos Chelén, pers. observ.). Our data also resemble the findings of Adhikerana and Slater (1993) with coal tits (*Parus ater*), which suggested that long songs are perceived as a more threatening signal than shorter songs. They also evidenced that coal tits respond to an increasing song rate as a more serious threat than to a decreasing song output. Although we found that vermilion flycatchers did respond to a high song rate before nest construction, we did not find a differential response to both treatments (i.e. high

and low song rate). It is puzzling that male vermilion flycatchers appear oblivious to variations in song rate, a song parameter that has been correlated with level of arousal and escalated male-male interactions (Naguib 1999, review in Vehrencamp 2000). On the other hand, it may be that males are not unaware of song rate but that our sample is too small to detect an effect, or that we assessed unsuitable response variables.

However, vocalization rate increased with playback of a male song, thus seeming biologically relevant. It is still also possible that song rate in the studied species is relevant in inter-sexual contexts only, perhaps as a signal of condition and food availability in the territory to potential mates (Alatalo et al. 1990, Cuthill & Macdonald 1990).

We conclude that male vermilion flycatchers are able to differentiate between long and short songs, and interpret our results as long songs being more threatening signals than shorter ones. Finally, given the large variance in the responses evoked by the play backs, it becomes relevant to evaluate the effect of other song features that correlates with song length such as number of introductory elements and length of terminal element (Ríos Chelén et al. 2005) on the territorial responses of breeding males.

### **Acknowledgements**

We want to thank R. A. Lucio and M. Martínez of the Centro Tlaxcala de Biología de la Conducta, UAT, for allowing the use of the laboratory facilities during field work. A. Guillén, L. D'Alba, C. Espinoza, and C. Ibarra greatly assisted during

field work and A. Aebi assisted in adjusting the amplitude of the playbacks. We thank K. Riebel and K. Renton for commenting on a previous version of the manuscript, and N. Geberzahn for help with the statistical analyses. We thank the Behavioural Biology group at Leiden University for discussion on the playback design, especially C. ten Cate, K. Riebel, A. Leitão, S. de Kort, G. Beckers and R. Lachlan, and the Lunáticos Behaviour Discussion Group for constructive feedback, especially Carlos Cordero. AARCH was supported by a CONACyT PhD and a PAEP (project #201318) grants and CMG with a DGAPA (UNAM) research grant. We are grateful to two anonymous referees that improved this manuscript with feedback on the methods and analyses.

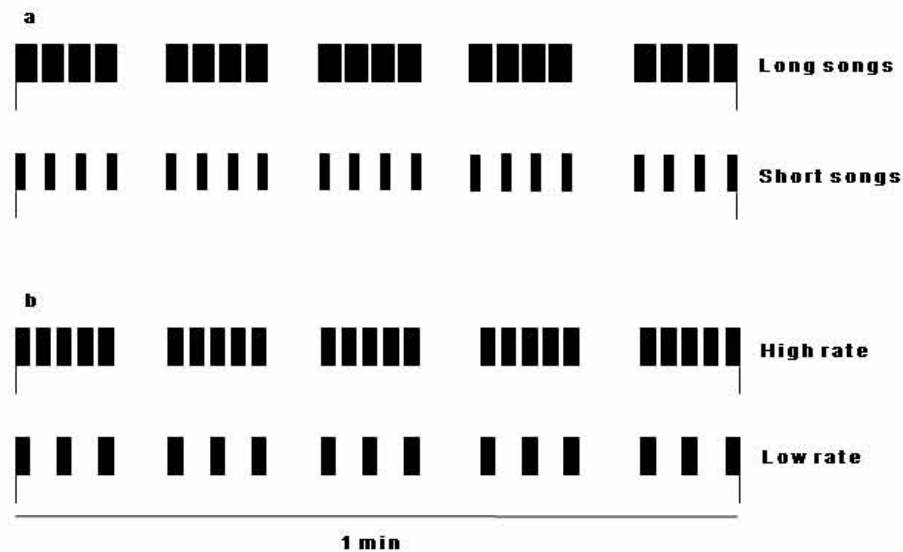


Fig. 1 Each male was exposed to each experiment (a and b). a) Song length: during 1 min of playback males were exposed to long (7 or 8 introductory elements) or short (3 or 4 introductory elements) songs. b) Song rate: males were exposed to a high (25 songs/min) or low (15 songs/min) song rate playback. Each rectangle represents a song. The figure represents the playback min only, the pre- and post playback min (silence) are not shown.

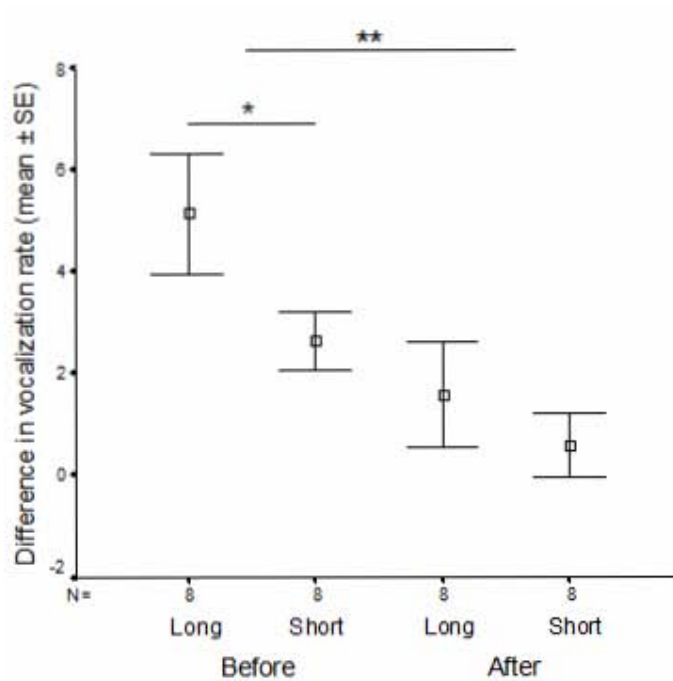


Fig. 2 Males vocalized more in response to long songs than to short songs before nest construction, but not after the onset of nest construction. A line with an asterisk is shown to emphasize this difference. Overall vocalization response before nest construction (both considering response to long and short songs) was higher than after nest construction. A line with two asterisks is shown to emphasize this difference. Before = before nest construction. After = after nest construction. Long = long songs. Short = short songs. See Table 2 for statistics.



Table 1. Results of Friedman related samples test showing significant responses of birds in different moments of playback experiment (i.e. pre-, play- and post playback). BNC = before nest construction, ANC = after nest construction. Numbers in bold indicate significant result after Bonferroni correction. In all significant instances this meant that bird's activity rate was higher during the playback minute than during the pre playback minute of silence, and also that birds' measured behaviour did not differ between the post playback minute and the pre- and playback minutes. Post hoc comparisons with Wilcoxon-Wilcox test for related samples.

Treatment	Measured behaviour			
	Calls	Songs	Vocalizations	Flights
BNC Long	$\chi^2 = 12, \mathbf{P} = 0.002, N = 12$	$\chi^2 = 6, P = 0.039, N = 12$	$\chi^2 = 13, \mathbf{P} = 0.001, N = 12$	$\chi^2 = 6, P = 0.047, N = 8$
BNC Short	$\chi^2 = 12, \mathbf{P} = 0.002, N = 12$	$\chi^2 = 2, P = 0.368, N = 12$	$\chi^2 = 12, \mathbf{P} = 0.002, N = 12$	$\chi^2 = 4, P = 0.093, N = 8$
BNC High	$\chi^2 = 10, \mathbf{P} = 0.005, N = 10$	$\chi^2 = 0, P = 1.0, N = 10$	$\chi^2 = 10, \mathbf{P} = 0.005, N = 10$	$\chi^2 = 6, P = 0.032, N = 8$
BNC Low	$\chi^2 = 7, P = 0.025, N = 10$	$\chi^2 = 2, P = 0.368, N = 10$	$\chi^2 = 7, P = 0.023, N = 10$	$\chi^2 = 3, P = 0.158, N = 8$
ANC Long	$\chi^2 = 10, \mathbf{P} = 0.006, N = 11$	$\chi^2 = 2, P = 0.368, N = 11$	$\chi^2 = 10, \mathbf{P} = 0.006, N = 11$	$\chi^2 = 7, P = 0.021, N = 11$
ANC Short	$\chi^2 = 3, P = 0.223, N = 11$	$\chi^2 = 2, P = 1.0, N = 11$	$\chi^2 = 3, P = 0.223, N = 11$	$\chi^2 = 2, P = 0.368, N = 11$
ANC High	$\chi^2 = 2, P = 0.313, N = 10$	$\chi^2 = 2, P = 0.368, N = 10$	$\chi^2 = 1, P = 0.539, N = 10$	$\chi^2 = 4, P = 0.118, N = 9$
ANC Low	$\chi^2 = 8, P = 0.018, N = 9$	$\chi^2 = 0.2, P = 0.905, N = 9$	$\chi^2 = 6, P = 0.050, N = 9$	$\chi^2 = 0.5, P = 0.747, N = 8$

Table 2. Results of Two Way Repeated Measures ANOVA for vocalizations as response variable to playbacks of long songs (long) and short songs (short), for different periods of the breeding cycle (i.e. before and after the onset of nest construction).

Source of Variation	DF	F	P
Breeding period	1	14.304	<b>0.007</b>
Song length	1	4.667	0.068
Breeding period x song length	1	1.432	0.270
Tukey Post hoc test			
Before vs after nest construction			<b>0.007</b>
Long vs short songs			0.068
Before nest construction, long vs short songs			<b>0.030</b>
After nest construction, long vs short			0.347
Long songs, before vs after nest construction			<b>0.003</b>
Short songs, before vs after nest construction			0.053

### Literature cited

- Adhikerana, A.S & Slater, P.J.B. 1993: Singing interactions in coal tits, *Parus ater*: an experimental approach. *Anim. Behav.* **46**, 1205-1211.
- Alatalo, R.V.; Glynn, C. & Lundberg, A. 1990: Singing rate and female attraction in the pied flycatcher: an experiment. *Anim. Behav.* **39**, 601-603.
- Archer, T.J. 1996: Observations on nestling and display flights of the vermilion flycatcher in western Texas. *Southwestern Naturalist.* **41**, 443-444.
- Ballentine, B.; Badyaev, A. & Hill G.E. 2003: Changes in song complexity correspond to periods of female fertility in blue grosbeaks (*Guiraca caerulea*). *Ethology*, **109**, 55-66
- Balsby, T.J.S. & Dabelsteen, T. 2001: The meaning of song repertoire size and song length to male whitethroats *Sylvia communis*. *Behavioural Processes.* **56**, 75-84.
- Catchpole, C.K.; Leisler, B. & Dittami, J. 1986: Sexual differences in the responses of captive great reed warblers (*Acrocephalus arundinaceus*) to variation in song structure and repertoire size. *Ethology.* **73**, 69-77.
- Catchpole, C.K. 2000: Sexual selection and the evolution of song and brain structure in *Acrocephalus* warblers. *Adv. Study Behav.* **20**, 45-97.
- Cuthill, I.C. & Macdonald, W.A. 1990. Experimental manipulation of the dawn and dusk chorus in the blackbird *Turdus merula*. *Behav. Ecol. Sociobiol.* **26**, 209-216.
- De Benedictis, P. 1966: The flight song display of two taxa of vermilion flycatcher, genus *Pyrocephalus*. *Condor* **68**, 306-307.
- Hoelzel, A. R. 1986: Song characteristics and response to playback of male and female robins *Erithacus rubecula*. *Ibis.* **128**; 113-127

- Ikebuchi, M.; Futamatsu, M. & Okanoya, K. 2003: Sex differences in song perception in Bengalese finches measured by the cardiac response. *Anim. Behav.* **65**, 123-130.
- Kroodsma, D.E. 1984: Songs of the Alder Flycatcher (*Empidonax alnorium*) and the Willow Flycatcher (*Empidonax traillii*) are innate. *Auk* **101**, 13-23.
- Langemann, U.; Tavares, J.P.; Peake, T.M. & McGregor, P.K. 2000: Response of great tits to escalating patterns of playback. *Behaviour.* **137**, 451-471.
- Leitão, A. & Riebel, K. 2003. Are good ornaments bad armaments? Male chaffinch perception of songs with varying flourish length. *Anim. Behav.* **66**, 161-167.
- Lothar, S. 1997: *Angewandte statistik. Anwendung statistischer methoden.* Springer-Verlag. Berlin.
- Lovell, S. F. & Lein, R. 2004: Neighbor-stranger discrimination by song in a suboscine bird, the alder flycatcher, *Empidonax alnorum*. *Behav. Ecol.* **15**, 799-804.
- Mace, R. 1987: The dawn chorus in the great tit *Parus major* is directly related to female fertility. *Nature.* **330**, 745-746.
- McGregor, P. K. & Horn, A.G. 1992: Strophe length and response to playback in great tits. *Anim. Behav.* **43**, 667-676.
- Møller, A.P. 1991: Why mated songbirds sing so much: mate guarding and male announcement of mate fertility status. *American Naturalist* **138**, 994-1014.
- Naguib, M. 1999. Effects of song overlapping and alternating on nocturnally singing nightingales. *Anim. Behav.* **58**, 1061-1067.
- Rice, W.R. 1989: Analyzing tables of statistical tests. *Evolution* **43**, 223-225.
- Ríos-Chelén, A. A. & Macías-García, C. 2004. Flight display song of the Vermilion Flycatcher (*Pyrocephalus rubinus*). *Wilson Bulletin.* **116**, 360-362.
- Ríos Chelén, A. A.; Macias-García, C. & Riebel, K. (2005). Song variation in a sub-

- oscine bird, the vermilion flycatcher (*Pyrocephalus rubinus*). Behaviour. 142, 1121-11138.
- Smith, W.J. 1967: Displays of the vermilion flycatcher (*Pyrocephalus rubinus*). Condor. **69**, 601-605.
- Smith, W.J. & Smith, A.M. 1996: Vocal signalling of the Great Crested Flycatcher, *Myiarchus crinitus* (Aves, Tyrannidae). Ethology. **102**, 705-723.
- Vehrencamp, S.L. 2000. Handicap, index, and conventional signal elements of bird Song. In: Animal signals: signalling and signal design in animal communication, pp. 277-300. Ed. By Y. Espmark, T. Amundsen, G. Rosenqvist. Tapir Academic Press, Trondheim, Norway.
- Wasserman, F.E. & Cigliano, J.A. 1991: song output and stimulation of the female in white-throated sparrows. Behav. Ecol. Sociobiol. **29**, 55-59.

**Extra-pair reproduction  
and song length in  
the vermilion flycatcher**

## **Extra-pair reproduction and song length in the vermilion flycatcher**

Alejandro Ariel Ríos Chelén<sup>1,2</sup>, Jefferson A. Graves<sup>3</sup>, Roxana Torres<sup>1</sup>, Miguel Serrano Pinto<sup>1</sup>, Liliana D'Alba<sup>1,4</sup> and Constantino Macías García<sup>1</sup>

<sup>1</sup>Departamento de Ecología Evolutiva, Instituto de Ecología, UNAM, AP 70-275, CP 04510, Mexico D.F., Mexico. <sup>3</sup>School of Biology, University of St. Andrews, KY169TS, Scotland, UK. <sup>4</sup>IBLS, Division of Environmental & Evolutionary Biology, University of Glasgow, Scotland, UK

<sup>2</sup>Corresponding author's email address: [aarios@miranda.ecologia.unam.mx](mailto:aarios@miranda.ecologia.unam.mx)

Corresponding author's telephone: (55) 56 22 9044

Corresponding author's fax: (55) 56 16 1976

It has been suggested that extra-pair paternity plays an important role in sexual selection and the evolution of sexual dimorphism in monogamous species. In this study we asked whether extra-pair reproduction exists in the sexually dimorphic and socially monogamous vermilion flycatcher (*Pyrocephalus rubinus*), and if so, whether some males are able to increase their reproductive success through extra-pair copulations. Males sing longer songs when their mates are potentially fertile, and vocalized more in response to long than to short songs when these songs were played back simulating an intruder in the male's territory. We also tested the hypothesis that long songs are used as a paternity guarding signal in this species. We predicted that males singing longer songs should be less cuckolded, and have higher reproductive success. We found that both extra-pair paternity and brood parasitism occur in this species, but there was no significant relationship between male song length and any aspect of extra-pair reproduction. We did find that some males may benefit from extra-pair reproduction since variance in male reproductive success was more than doubled in one year (2.79-fold), as a result of extra-pair matings. We discuss these findings and suggest some possible functions of the song of the vermilion flycatcher.

*Key words: extra-pair reproduction, Vermilion Flycatcher, Pyrocephalus rubinus, sub-oscine, song length, intra-specific brood parasitism*



## INTRODUCTION

Several studies have demonstrated the importance of sexual selection in the evolution of different vocal attributes in birds (reviewed in Catchpole and Slater 1995). Most have focused on the differential response of males and females to different song variants (e.g. different repertoire sizes) and on how reproductive success correlates with particular vocal characteristics. While the majority of birds have been considered to be monogamous, there is an increasing number of studies showing the occurrence of extra-pair paternity in socially monogamous species (reviewed in Griffiths et al. 2002). Therefore, any study investigating the relationship between vocal behaviour and reproductive success must take into account that extra-pair paternity is a possibility that can increase the variance in reproductive success between individuals, and results from studies comparing only within-nest reproductive success should be “treated with caution” (Delhey et al. 2003).

While theory predicts intense sexual selection resulting in marked sexual dimorphism in polygamous species, sexual selection resulting in sexual dimorphism in monogamous species is less well understood. Birkhead and Møller (1992) proposed that differences in reproductive success among individual males resulting from differences in the rate of extra-pair paternity (EPP) drives the evolution of sexual dimorphism in monogamous species. If the variance in reproductive success among individuals is greater when EPP occurs than the variance in the absence of EPP, then the extra-pair paternity hypothesis is supported. Yezerinac et al. (1995) found in the yellow warbler, (*Dendroica petechia*), a socially monogamous and sexually dimorphic species, that extra-pair paternity resulted in some males being more successful than others, and the variance in reproductive

success was greater than that expected from assuming that the male fathered the offspring in his nest only. Hence, if extra-pair paternity promotes variation in reproductive success among individuals, it can play an important role in the process of sexual selection. A differential reproductive success via EPP has also been found in purple martins (*Progne subis*) where older males gain a higher proportion of extra-pair fertilizations (EPF) (Morton et al. 1990, Wagner et al. 1996a, Wagner et al. 1996b). In this case high levels of EPF have been associated with the evolution of colonial breeding.

Another line of research has focused on the benefits females may obtain from extra-pair copulations. Females may be constrained by male availability when selecting a breeding partner (i.e. the most attractive males are mated early in the season), and thus the available males for late breeding females may be less attractive (Møller 1992). Therefore, females may redress this sub-optimal mating by seeking extra-pair copulations. The benefits a female may obtain from mating with an extra-pair male are either indirect (e.g. genetic benefits) or direct (e.g. gaining extra-food from the extra-pair male). So far there is more evidence for indirect benefits (e.g. Kempenaers et al. 1992, Hasselquist et al. 1996, Kempenaers et al. 1997, but see Schmoll et al. 2003).

Although both sexes may obtain benefits from extra-pair copulations, the relationship between extra-pair paternity and sexual selection is not always clear; some studies have explained the variation in sexual dimorphism as closely linked to EPP, while others have found no apparent correlation between EPP and male traits. In a comparative study, Møller and Birkhead (1994) showed that the degree of sexual dichromatism is positively correlated with EPP, suggesting that EPP has played an important role in the process of sexual selection acting on the evolution of plumage brightness in birds. Other

studies, however, have failed to detect any correlation between EPP and plumage coloration or other male morphological traits (Hill et al. 1994, Dunn et al. 1994, Rätti et al. 1995).

The vermilion flycatcher (*Pyrocephalus rubinus*), is a socially monogamous, and sexually dimorphic sub-oscine. Females build the nests and incubate the clutch of 1 to 4 eggs (usually 3) and both parents feed the young (Días Ríos 2002, Ríos Chelén pers. obs.). The bright red plumage on the chest and head of the males is very different from the female's plumage. Sexual dimorphism is also related to the singing behaviour since females do not usually sing (i.e. 3 females were seen singing once in the years 2000 and 2003). Males, on the other hand, have a peak of singing activity at dawn. It has been previously demonstrated that the greatest variation in song among males is contained in length-related traits (Ríos Chelén, et al. 2005). Males sing longer songs after the onset of nest construction, when their partner is potentially fertile, than in the period before the onset of nest construction, and males vocalized more to long than to short songs in a playback experiment (Ríos Chelén and Macías Garcia unpublished data). Natural observations on agonistic encounters plus the recorded behaviours in the playback experiment, where we simulated intrusions by a potential intruder, clearly showed that vocalizing was indeed a behaviour associated with aggressive interactions. Although we did not find that males singing longer songs are more efficient at repelling intruders, these results suggest that longer songs may act as a paternity guarding signal. Consequently we hypothesised that long songs could be used as paternity guarding signals in this species. If so, we would predict that males singing longer songs would suffer less paternity losses in their own nests, and would be more successful.

Here we studied: 1) whether EPP is common in this species, and if so, 2) whether the variance in reproductive success among males is higher when considering EPP than in the absence of EPP, 3) whether males singing longer songs are less cuckolded, and 4) whether or not song length correlates with overall reproductive success in this species.

## STUDY AREA

We studied a population of vermilion flycatchers ( $n =$  around 24) located in the Forest of San Diego Metepec ( $19^{\circ} 17.97$  N,  $98^{\circ} 14.60$  W), Tlaxcala, Mexico, where blood samples and song recordings were collected in 2001 and 2003. The study site is a mixture of native *Pinus* spp and introduced *Eucalyptus* spp forest and open areas. Most vermilion flycatchers in this population are migrants, with the exception of 2 males that were evidently year round residents. The reproductive season for this population starts around February (when migrant males start to establish territories), and finishes around late July or early August (when most males are gone).

## MATERIALS AND METHODS

### **Songs analysis**

Songs were recorded at and before dawn, from around 0530, when it became apparent that males started to sing, until 0630 hrs, when males began to call and forage. Songs were

recorded with a Sennheiser ME66 microphone and a Marantz PMD221 cassette recorder at two different periods in the breeding season, before and after the onset of nest construction. Songs with little background noise were digitized on a PC computer using either a Yamaha® Sound card DS-XG PCI audio (WDM) or a Creative Audio® PCI (ES 1371, ES 1373 [WDM]) sound card. Songs were high-pass filtered with a cut-off frequency below 2.8 kHz, and sampled at a rate of 22050 Hz using the Avisoft® SASLabPro software. We used 10 songs per individual, 5 recorded before nest construction commenced, and 5 recorded after the onset of nest construction. These were randomly chosen from the set of digitized songs. In a previous study (Ríos Chelén, 2005), it was found that aspects related to song length showed the greatest variation among individuals, and also that males sing longer songs after nest construction begins than in the period previous to it. Therefore, we focused our attention in measuring the length of the songs. This was done manually with the on-screen cursor available in Avisoft® SASLabPro software.

### **Blood samples and DNA analysis**

Adults were captured with mist nets and baited spring traps and colour ringed. Chicks were collected at their nests when 8 days old. Blood samples (70-240µl) were taken by puncturing the brachial vein, and collecting the blood with capillary tubes. Samples were stored in lysis buffer at 4 °C until DNA was extracted.

DNA was obtained by phenol-chloroform extraction and ethanol precipitation (Sambrook et al 1989). We used 3 micro-satellites developed for this species by M. Serrano, J. A. Graves, C. Macías Garcia and R. Torres. Micro-satellites 448, 274, and 390 have seven, four, and two alleles respectively. This gave a total exclusionary power of 51.5

and 43.5% for all three microsatellites combined in years 2001 and 2003 respectively, and a mean polymorphic information content (PIC) of 0.50 and 0.44 for years 2001 and 2003 respectively (PIC is a measure on the proportion of heterozygous individuals and allele frequencies in a population; formula in Hearne et al., 1992); thus our assessments of extra-pair paternity must be regarded as quite conservative. Approximately 3-10ng of genomic DNA was amplified in 10 $\mu$ L of PCR reactions containing 1x buffer, 1.5mM MgCl<sub>2</sub> (for micro-satellites 274 and 390) or 1.0 mM MgCl<sub>2</sub> (for micro-satellite 448), 10pM of each primer, 0.5U of *Taq* and made up to volume with sterile distilled water. The reactions were denatured at 95°C for 4 minutes followed by 30 cycles of 95°C for 10 seconds, primer-specific annealing temperatures (60, 54 or 56°C for micro-satellites 448, 390 and 274 respectively) for 45 seconds, 72°C for 45 seconds and final elongation at 72°C for 5 minutes. PCR products were separated on 6% polyacrylamide gels, visualized by silver staining, and allele lengths scored from a 10 base pair DNA ladder (Invitrogen).

### **Statistical analyses**

Since one of the objectives of this study was to determine whether or not there is an association between song length and reproductive success, we entered in the analysis all males and chicks from which we had DNA samples. There were 8 males (6 families plus two local males), and 18 chicks in 2001; and 13 males (8 families plus five local males), and 21 chicks in 2003 (Table 1a). A local male is one that was not observed continuously during the study, and whose breeding status was not determined, or a male whose female was not seen to build a nest. Paternity analyses were done with Cervus version 2 (Marshall T. 2001).

We counted the number of offspring assigned to each father, and used it as a measure of reproductive success. Since we did not have recordings of songs from both periods for all males, our sample was slightly different for the different periods. Therefore, we performed simple correlations for each of the three different conditions in which song was recorded (1 = before, 2 = after nest construction, and 3 = the average of both periods) and later corrected the P-value considered for significance with a Sequential Bonferoni Correction (Rice 1989).

Three adult males were sampled in both 2001 and 2003. The analyses with Cervus were performed year by year with all males and chicks. However, to evaluate the correlations between our measure of reproductive success and song length, we took care that no individual was represented more than once in the analyses. To this end we assessed the correlations twice whenever necessary, considering the reproductive performance of the repeated individuals in 2001 and 2003 separately. Whenever multiple tests were carried out using the same data set we adjusted the  $\alpha$  level with sequential Bonferoni corrections. All correlations were two-tailed.

## RESULTS

### **Extra-pair reproduction**

In 2001, one out of six nests (16.6%) had chicks with extra-pair paternity, and 4 (23.5%) chicks were extra-pair. From these, 3 chicks (17%) resulted from extra-pair paternity. The other chick was not the offspring of the female on the nest, but was of the male (i.e. quasi-

parasitism, Yezerinac et al 1995). In 2003, three out of eight families (37%) were found to have extra-pair offspring, and eight chicks (38%) were extra-pair. From these, two families (25%) had at least one chick that resulted from extra-pair paternity, and three out of twenty one chicks (14%) resulted from extra-pair paternity. In three families we found evidence of intra-specific brood parasitism (i.e. neither the male nor the female were the parents). As in the previous year we found one chick whose mother was not the female on the nest (i.e. quasi-parasitism). The female in family 6B was also seen in family 11, and therefore considered this to be a case of polyandry (Table 1a), though we could not be certain that this female fed the young in family 11.

### **Reproductive success within nests, and song length**

There was no correlation between the number of chicks that were fathered by the nest male and his song length. This was also the case when the analysis included the data in 2001 or 2003 of the three males that were sampled in both years (Table 2).

In relation to the percentage of extra-pair offspring within nests and song length, we did not find any significant correlation between these two variables, regardless of whether we analysed the repeated males in 2001 or 2003 only, and whether we used song recorded before or after nest construction, or when both moments were averaged ( $P > 0.3$  in all correlations). Additionally, we did not find any significant correlation between the percentage of offspring resulted from extra-pair maternity and the male's song length, nor when we used songs recorded before or after nest construction, or when both periods were averaged ( $P > 0.85$  in all instances).



### **Reproductive success, as percentage of offspring sired by each most likely male, and song length**

Cervus assigned a most likely parent at the 24 and 25% level of confidence (for 2001 and 2003 respectively) in 36 of the 39 chicks.

We had two males repeated in both years, and lacked songs before nest construction in one of the years (2003) for one of them. There was no significant correlation between the percentage of offspring sired by the likely father, and its song length, when we included the repeated males in the year 2001 only. This was the case irrespective of whether we used songs recorded before or after nest construction, or when songs lengths were averaged for both periods ( $r = 0.15$ ,  $P = 0.66$ ,  $n = 10$ ;  $r = 0.28$ ,  $P = 0.42$ ,  $n = 10$ ;  $r = 0.27$ ,  $P = 0.44$ ,  $n = 10$ ; respectively).

When the repeated males were included in the 2003 data only, we found a non-significant tendency of males singing longer songs, before the onset of nest construction, to sire a higher proportion of offspring ( $r = 0.61$ ,  $P = 0.057$ ,  $n = 10$ ). This non-significant tendency was far away from significant after Bonferroni Correction ( $\alpha = 0.008$ ). There was no significant correlation between percentage of offspring sired by each most likely father and its song length, when we used songs recorded after nest construction ( $r = 0.08$ ,  $P = 0.82$ ,  $n = 10$ ), or when data in both periods were averaged ( $r = 0.45$ ,  $P = 0.19$ ,  $n = 10$ ).

### **Variance in male mating success: reproductive success resulted from within-pair reproduction and EPP, as opposed to within-pair reproduction alone**

Given the mosaic of different events of extra-pair reproduction (i.e. from extra-pair paternity to intra-specific brood parasitism) found in this study, we asked whether the

variance in reproductive success among males is higher via EPP than that expected from within-pair reproduction alone. Following Yezerinac et al. (1995), we calculated the relative variance ( $I_s$  = variance in reproductive success divided by the square of mean success) in both apparent and actual success. Apparent success was the number of offspring in the male's nest, while actual success was the number of offspring sired in any nest. Relative variance in actual success was divided by relative variance in apparent success to obtain the increase in differential reproductive success among males that could result from within-pair and EPP over within-pair reproduction alone. Relative variance was obtained for two data sets separately. The first set included both males that gained and those that lost paternity to extra-pair matings. Because this set contained both males that gained ("winners") and lost ("losers") extra-pair paternity, it provides an upper limit of variance in reproductive success that could result from within-pair and extra-pair reproduction. The second set included only those males known to have gained extra-pair offspring (EPO). Because this data set comprised only "successful" males, it provides a lower limit for variance in male mating success (see Yezerinac et al 1995). For these analyses we did not combine individuals that were repeated in both years (i.e. 2001 and 2003). Therefore these analyses were carried out twice, once with repeated individuals represented in 2001 only, and once with repeated individuals represented in 2003 only. As shown in Table 3, the variance in reproductive success calculated this way increased up to 1.64-fold, or 2.79-fold (for those repeated males represented in 2001 only, or 2003 only respectively), over that which would be expected from within-pair reproduction alone.

## DISCUSSION

Over the two years, we found that 16-25% of the nests contained extra-pair offspring resulted from extra-pair paternity, and 17-14 % of the chicks in those nests resulted from extra-pair paternity. This is well within the range of extra-pair paternity found in other species, where it has been shown that the levels of extra-pair paternity can range from 0 to more than 70% (Møller and Birkhead 1994). However, the rates of EPP found in socially monogamous passerines are in general lower than 70%, with high levels of 25% or up to 55% for the reed bunting (*Emberiza schoeniclus*) (Griffith et al. 2002). Extra-pair reproduction can take several forms (Yeserinac et al. 1995). Despite our small sample size, we found that the reproductive system of the vermilion flycatcher is more complicated than simple monogamy, and that the cases of extra-pair reproduction found in this species go from offspring where the putative father is not the genetic father, or the male is but the female is not the genetic mother (quasi-parasitism) to cases where neither the male nor the female are the parents (intra-specific brood parasitism). While there have been reports of cases of inter-specific brood parasitism in the vermilion flycatcher (Friedmann 1963), this is the first time that intra-specific parasitism has been reported in this species. Intra-specific brood parasitism has been reported in numerous other species (Birkhead et al 1990, McKittrick 1990, Petrie and Møller 1991, Jackson 1993, McRae and Burke 1996, Lyon 2003), but associations with phenotypic male attributes are still lacking.

EPP can result from either forced or unforced extra-pair copulations, as well as through rapid mate switching (Birkhead et al 1990). Since most bird species lack an intromittent organ (Birkhead and Møller 1992) females must, to some extent, consent to

participate in extra-pair copulations (Kempnaers et al 1997). During the years we studied this vermilion flycatcher population (2000-2003), we found that males frequently trespassed into the territories of other males during dawn or early morning. At least four different males were caught or seen in territories already occupied by other males. Since EPO was shown to occur, the purpose of these off-territory forays may have been to seek extra-pair copulation. However, more detailed behavioural studies are required before we can ascertain to what extent the observed patterns of EPP are driven by male or female movements off territory and what are the benefits of males and females of extra pair matings.

A correlation between song and EPP was expected in the view of previous findings. Kempnaers et al. (1992) showed that female blue tits (*Parus caeruleus*) select males for extra-pair copulations on the basis of a particular song attribute: strophe length. Similarly, Hasselquist et al. (1996) found that female great reed warblers (*Acrocephalus rubinus*) prefer males with large song repertoires for extra pair fertilization, while Forstmeier et al. (2002) showed that in dusky warblers (*Phylloscopus fuscatus*), song amplitude correlates with EPP. In all these studies, females appeared to seek indirect benefits (i.e. genetic benefits) from extra-pair fertilizations. In our study, we did not find a correlation between the ratio of extra-pair offspring in a nest and song length. Assuming that the observed frequency of EPP is produced by females “waiting” for extra-pair males to enter in their territory, this negative result does not support the paternity guarding hypothesis.

The probability of assigning the most likely true father to each offspring was 24-25% (see results). This low probability was largely the result of the low proportion of males sampled in the population (40% in 2001 and 65% in 2003), and the low number of alleles

contained in the three micro-satellites (see methods). Since the power of our analyses is low, a larger sample using more polymorphic markers might find a correlation between song length and percentage of offspring sired by each most likely father.

We found an increased variance in male reproductive success via EPP over the variance based on simply assigning each chick to the male of the nest in which it hatched. This suggests that extra-pair reproduction may play an important role in the process of sexual selection. This variance ranged from 0.16-1.64-fold and/or 0.006-2.79-fold times (see results) over the variance that would be expected from within-pair reproduction alone. Yezerinac et al. (1995) found that male yellow warblers (*Dendroica petechia*) also benefit from extra-pair fertilizations as they increase their variance in reproductive success, but between 3-fold and 15-fold over that which would be expected from within-pair reproduction alone. Our results on the variance in male mating success with EPP are conservative, since our estimates of extra-pair parentage were conservative.

In this species there is no evidence that the attributes that convey information about the male quality to females seeking extra-pair copulations, are related to the song characteristic that we measured. Møller and Birkhead (1994), and Garamszegi and Møller (2004) performed comparative analyses that showed that plumage dichromatism, but not song, is related to EPP, which suggests that plumage coloration is an important target of sexual selection in many species, while song attributes appear to have played a less important role in promoting variation in reproductive success via EPP. The evidence of EPP presented in this work, plus the fact that vermilion flycatchers are sexually dichromatic, makes variation in plumage colouration a likely candidate to look for a possible target of sexual selection via extra-pair copulations.

In conclusion, we found instances of extra-pair reproduction in the vermilion flycatcher, and this included intra specific brood parasitism. The observed frequency of extra-pair reproduction, plus the increased variance in male reproductive success via EPP, calls for further studies to assess which morphological (e.g. body mass and size, plumage colouration) or behavioural attributes (e.g. courtship) promote paternity via extra-pair reproduction.

#### AKNOWLEDGEMENTS

We thank Rosa Angélica and Margarita Martínez, from the Centro Tlaxcala de Biología de la Conducta from the Universidad Autónoma de Tlaxcala for allowing the use of the laboratory facilities during field work. Alejandro Guillén, Cristina Espinosa, and Carlos Ibarra greatly assisted during field work. We thank Haydé Peralta for assisting in measuring the songs from the year 2003, and Carlos Cordero and Katherine Renton for sharing with us discussions on this study. AARCH was supported by a CONACyT PhD and a PAEP (project #201318) grants and CMG with a UNAM (DGAPA) research grant (PAPIIT IN225799).

Table 1a. Paternity data on vermilion flycatchers (years 2001 and 2003).

2001		2003	
FAMILY	Extra-pair young?	FAMILY	Extra-pair young?
1A		1B	
	Chick 1		Chick 1
	Chick 2		Chick 2
	Chick 3	5B	
	Chick 4		Chick 1
	Chick 5		Chick 2
2			Chick 3
	Chick 1 EPP	6B	
	Chick 2 EPP		Chick 1 ISBP
	Chick 3 EPP		Chick 2 ISBP
3			Chick 3 EPP
		7	
	Chick 1		Chick 1 ISBP
	Chick 2 QP		Chick 2 QP
4		8	
	Chick 1		Chick 1
	Chick 2		Chick 2
5A		9	
	Chick 1		Chick 1

---

	Chick 2		Chick 2
6A			Chick 3
	Chick 1	10	
	Chick 2		Chick 1 EPP
	Chick 3		Chick 2 EPP
			Chick 3 IPBP
		11	
			Chick 1
			Chick 2
			Chick 3

---

Family 1A (in year 2001) had 5 chicks from two reproductive attempts (two chicks in one, three in the other). Additionally to the individuals shown in this table, we had data from other potential parents in the population: families 12 (male and female), 13 (female), 14 (female), and 15A (male) from year 2001; families 15B (male and female), 16 (male and female), 17 (male and female), 18 (male), and 19 (male) from year 2003. Letters A and B refers to a male that was repeated in both 2001 and 2003 years (e.g. family 15A in year 2001 had the same male as in family 15B in year 2003). EPP = extra-pair paternity, the male on the nest is not the genetic father, QP = quasi-parasitism, the female on the nest is not the genetic mother, ISBP = intra-specific brood parasitism, nor the male nor the female on the nest are the genetic parents.



Table 1b. Individuals from which we lack DNA.

2001	2003
Family 3, chick 3	Family 1B, female
Family 4, female	Family 5B, female
Family 5A, female	Family 8, female
Family 6A, female	

Table 2. Song length and within nest reproductive success.

	First analysis			Second analysis		
	n	<i>r</i>	<i>P</i>	n	<i>r</i>	<i>P</i>
Before nest construction	11	-0.207	0.271	10	0.067	0.427
After nest construction	11	0.359	0.279	11	0.046	0.447
Both moments averaged	11	0.359	0.279	10	0.091	0.401

Analyses were performed using songs recorded before nest construction, after nest construction or using the average of the two periods. Since three males were sampled in both, years 2001 and 2003, we performed the analyses twice: the first analysis included the repeated males as data points in the year 2001 only, and the second analysis as data points in the year 2003 only. When  $n = 10$  we did not have songs for one male before nest construction.

Table 3. Relative variance in reproductive success ( $I_s$ ) for both apparent and actual mating success. See text for details.

Repeated males represented in 2001 only					
Data set	n	Mean	Variance	$I_s$	Actual/apparent
All males					
Apparent	10	2.3	2.23	0.42	
Actual	10	2.6	4.71	0.69	1.64
Extra-pair fathers					
Apparent	5	1.4	1.8	0.91	
Actual	5	4.20	2.70	0.15	0.16
Repeated males represented in 2003 only					
Data set	n	Mean	Variance	$I_s$	Actual/apparent
All males					
Apparent	11	2.09	1.29	0.29	
Actual	11	1.72	2.41	0.81	2.79
Extra-pair fathers					
Apparent	3	0.66	1.33	3.0	
Actual	3	3.66	0.33	0.02	0.006

## REFERENCES

- Birkhead TR, Burke T, Zann R, Hunter FM, Krupa AP (1990) Extra-pair paternity and intraspecific parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting. *Behav Ecol Sociobiol* 27:315-324
- Birkhead TR, Møller AP (1992) Sperm competition in birds. Evolutionary causes and consequences. Academic Press. London
- Catchpole CK, Slater PJB (1995) Bird Song: Biological Themes and Variations. University Press. Cambridge
- Delhey K, Johnsen A, Peters A, Andersson S, Kempenaers B (2003) Paternity analysis reveals opposing selection pressures on crown coloration in the blue tit (*Parus caeruleus*). *Proc R Soc Lond* 207:2057-2063
- Días Ríos M. (2002) Inversión de machos y hembras en el cuidado parental del mosquero cardenalito *Pyrocephalus rubinus*. Tesis de Licenciatura. Facultad de Ciencias, UNAM
- Dunn OP, Raleigh JR, Michaud-Freeman D, Boag PT (1994) Extra-pair paternity in tree swallows: why do females mate with more than one male? *Behav Ecol Sociobiol* 35:273-281
- Forstmeier W, Kempenaers B, Meyer A, Leisler B (2002) A novel song parameter correlates with extra-pair paternity and reflects male longevity. *Proc R Soc Lond B* 269:1479-1485
- Friedmann HL (1963) in Wolf BO, Jones SL (2000) Vermilion flycatcher (*Pyrocephalus rubinus*). In: The Birds of North America. No. 484 (A. Poole and F. Gill eds)

- Garamszegi LZ, Møller AP (2004) Extra-pair paternity and the evolution of bird song. *Behav Ecol* 15:508-519
- Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Mol Ecol* 11:2195-2212
- Hearne CM, Ghosh S, Todd JA (1992) Microsatellites for linkage analysis of genetic traits. *TIG* 8:288-294
- Hasselquist D, Bensch S, von Schantz T (1996) Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature* 381:229-232
- Hill GE, Montgomerie R, Roeder C, Boag P (1994) Sexual selection and cuckoldry in a monogamous songbird: implications for sexual selection theory. *Behav Ecol Sociobiol* 35:193-199
- Jackson WM (1993) Causes of conspecific nest parasitism in the northern masked weaver. *Behav Ecol Sociobiol* 32:119-126
- Kempnaers B, Verheyen GR, Van den Broeck M, Dhondt A (1992) Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature* 357:494-496
- Kempnaers B, Verheyen GR., Dhondt A (1997) Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behav Ecol* 8:481-492
- Lyon BE (2003) Ecological and social constraints on conspecific brood parasitism by nesting female American coots (*Fulica americana*). *Jour Anim Ecol* 72: 47-60
- Marshall T (2001) Cervus. Version 2. University of Edinburgh

- McKittrick CM (1990) Genetic evidence for multiple parentage in eastern kingbirds (*Tyrannus tyrannus*). *Behav Ecol Sociobiol* 26:149-155
- McRae SB, Burke T (1996) Intraspecific brood parasitism in the moorhen: parentage and parasite-host relationships determined by DNA fingerprinting. *Behav Ecol Sociobiol* 38:115-129
- Møller AP (1992) Frequency of female copulations with multiples males and sexual selection. *American Naturalist* 139: 1089-1101
- Møller AP, Birkhead TR (1994) The evolution of plumage brightness in birds is related to extrapair paternity. *Evolution* 48:1089-1100
- Morton ES, Forman L, Braun M (1990) Extrapair fertilizations and the evolution of colonial breeding in purple martins. *Auk* 17:275-283
- Petrie M, Møller AP (1991) Laying eggs in others' nests: intraspecific brood parasitism in birds. *TREE* 6:315-320
- Rätti O, Hovi M, Lundberg A, Tegelström H, Alatalo RV (1995) Extra-pair paternity and male characteristics in the pied flycatcher. *Behav Ecol Sociobiol* 37:419-425
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223-225
- Ríos Chelén A, Macías Garcia C, Riebel K (2005) Variation in the song of a sub-oscine, the vermilion flycatcher. *Behaviour* 142:1121-1138
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual*. Cold Spring Harbor, NY
- Schmoll T, Dietrich V, Winkel W, Epplen JT, Lubjuhn T (2003) Long-term fitness consequences of female extra-pair matings in a socially monogamous passerine. *Proc R Soc Lond B* 270:259-264

Wagner RH, Schug MD, Morton ES (1996a) Confidence, actual paternity and parental effort by purple martins. *Anim Behav* 52:123-132

Wagner RH, Schug MD, Morton ES (1996b) Confidence-dependent control of paternity by female purple martins: implications for coloniality. *Behav Ecol Sociobiol* 38:379-389

Yezerinac SM, Weatherhead PJ, Boag PT (1995) Extra-pair paternity and the opportunity for sexual selection in a socially monogamous bird (*Dendroica petechia*). *Behav Ecol Sociobiol* 37:179-188

**Conclusiones  
generales**

## CONCLUSIONES GENERALES

El primer objetivo de este trabajo fue describir el canto del cardenalito (*Pyrocephalus rubinus*). Mostramos que el cardenalito produce dos "tipos" de cantos: el canto emitido desde perchas (sobre todo durante el coro del amanecer) y el producido durante el vuelo de despliegue. Ambas vocalizaciones son muy semejantes en estructura ya que se componen de los mismos elementos, sin embargo la mayoría de los cantos producidos durante el vuelo de despliegue tienen además un elemento "extra", conocido como elemento *peent*. Este elemento está asociado a interacciones entre machos y entre machos y hembras. La presencia del elemento *peent* en las vocalizaciones durante vuelo despliegue, aunada a la observación anecdótica de que en ocasiones los machos realizan el vuelo de despliegue aparentemente como respuesta a otro vuelo despliegue de un macho vecino, apoya la idea de que este despliegue es usado como "proclamación" o defensa del territorio.

Con respecto al canto emitido desde percha, detectamos variación considerable entre machos en atributos relacionados con la longitud del canto. Esta variación es quizá poco esperada si consideramos que el cardenalito es una ave sub-oscina que probablemente no aprende a cantar, y al mismo tiempo abre la posibilidad de que la selección sexual pueda actuar sobre atributos del canto. La variación inter-individual encontrada en los cantos emitidos desde perchas, más la presencia de elementos *peent* en algunos cantos emitidos en vuelo de despliegue, nos hace cuestionarnos acerca de la idea comúnmente aceptada de que las aves sub-oscinas no aprenden a cantar. En estos momentos sin embargo no tenemos datos contundentes para poder aceptar o rechazar la idea de que el cardenalito no aprende a cantar; sin embargo hemos mostrado "plasticidad" en la emisión de canto.

Además de la variación inter-individual, también encontramos variación a lo largo de la temporada reproductiva, lo cual se manifestó en una variación intra-individual: los machos emiten cantos más largos (agregando elementos introductorios) después de empezada la construcción del nido, es decir cuando la pareja es potencialmente fértil, que antes de la construcción. La variación temporal no se limitó a este aspecto estructural del canto sino que también abarcó otro aspecto, la tasa de canto: los machos también cantaron más después de la construcción del nido que antes de su construcción. El hecho de que los machos modifiquen, en estructura y desempeño, sus cantos en aparente relación con el periodo fértil de la hembra, sugiere que una posible función del canto del cardenalito es el resguardo de paternidad. En otras palabras, que los cantos largos, y una tasa de canto mayor, son señales más agresivas que cantos cortos y que una tasa de canto menor, y que por lo tanto, al ser usados durante el periodo fértil de la hembra, sirvan para evitar pérdida de paternidad en su propio nido.

Una vez observada esta variación en el canto, la siguiente pregunta a responder fue si los machos discriminan o no entre diferentes cantos. Es decir si la variación observada es biológicamente significativa para los machos o en términos más coloquiales: ¿esta variación le dice algo a los machos? Mediante un experimento de playback mostramos que los machos son capaces de discriminar entre cantos largos y cortos: los machos respondieron más fuertemente ante cantos largos que cortos lo que,



aunado a observaciones de interacciones agonísticas en la naturaleza, sugiere que los cantos largos son señales más agresivas que los cantos cortos. Por lo tanto, los resultados de esta tesis sugieren que el canto en sub-oscinos (p.e. en el cardenalito) podría tener las mismas funciones, implicadas en las interacciones macho-macho y macho-hembra, que en oscinos. Por ejemplo, la tendencia negativa encontrada entre la longitud de la primera parte del canto y la longitud de la segunda parte abre la posibilidad de que el canto sea un resultado de diferentes presiones de selección (i.e. intra- e inter-sexual) actuando sobre diferentes atributos, como se ha sugerido ya en una especie de ave oscina, el pinzón vulgar (*Fringilla coelebs*). Aunque este trabajo se ha enfocado más a las interacciones entre machos y ha aportado evidencia de que el canto es usado como una señal agonística (i.e. quizá para repeler intrusos o resguardar la paternidad) que podría tener repercusiones en el éxito reproductivo, no podemos descartar la idea de que el canto pueda ser usado también para atraer o estimular hembras.

Habiendo observado que la variación de canto observada en la naturaleza parece ser biológicamente relevante para los machos, la siguiente pregunta se relacionó con la posibilidad de que el éxito reproductivo se relacione de alguna manera con la manera en que los machos cantan. Usando microsatélites, encontramos que en esta especie hay reproducción extra-pareja (REP). La REP varió desde casos en que el pollo del nido no es hijo del padre putativo (i.e. paternidad extra-pareja), donde la hembra no es la madre (i.e. quasi-parasitismo), a casos donde ninguno de los dos es padre o madre del pollo en el nido (i.e. parasitismo intra-específico). No encontramos ninguna relación entre la longitud del canto y el éxito reproductivo (dentro de su propio nido o considerando la ocurrencia de paternidad extra-pareja) o la probabilidad de perder paternidad en su propio nido. De manera que no podemos concluir que los cantos son usados como señales para resguardar la paternidad. De cualquier forma, los resultados de los análisis de paternidad nos indican que la selección de pareja para el apareamiento puede seguir ocurriendo aún después de que se ha formado una pareja social (i.e. para obtener cópulas extra-pareja). Estudios usando radiotransmisores podrían dar luz sobre cuál es el papel del macho y de la hembra en la búsqueda de cópulas extra-pareja. Esto plantea interesantes preguntas, por ejemplo: ¿porqué, si los cardenalitos son socialmente monógamos, existe la paternidad extra-pareja en esta especie? ¿Qué beneficios obtienen las hembras o los machos al obtener cópulas extra-pareja? En este trabajo vimos que algunos machos podrían beneficiarse de la paternidad extra-pareja al incrementar su éxito reproductivo. Por otro lado, los motivos que promueven que una hembra incurra en cópulas extra-pareja probablemente tengan que ver con beneficios indirectos (e.g. para sus crías: beneficios genéticos) o directos (e.g. tener a un buen padre que alimente adecuadamente a sus hijos).

Además del canto, un atributo que es buen candidato para ser seleccionado por la vía de la paternidad extra-pareja podría ser la coloración de los machos. Esto podría explicar porqué en esta ave socialmente monógama se presenta un tan marcado dimorfismo sexual.