UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE ECOLOGÍA
Biología Evolutiva

LA SELECCIÓN SEXUAL EN LA LAGARTIJA *Crotaphytus dickersonae*

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

MELISSA PLASMAN

TUTORA PRINCIPAL DE TESIS: DRA. LAURA ROXANA TORRES AVILÉS
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MEXICO, D.F., FEBRERO, 2016

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UNAM
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Ciencias Biológicas

COORDINACIÓN

Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
Presente

Me permito informar a usted, que el Subcomité de Biología Evolutiva y Sistemática, en su sesión ordinaria del día 21 de septiembre de 2015, aprobó el jurado para la presentación de su examen para obtener el grado de **DOCTORA EN CIENCIAS**, del Posgrado en Ciencias Biológicas, de la alumna **PLASMAN MELISSA** con número de cuenta **511021376** con la tesis titulada **"SELEXIÓN SEXUAL EN LA LAGARTIJA *Crotaphytus dickersonae*", bajo la dirección de la DRA. LAURA ROXANA TORRES AVILÉS:**

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Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARA EL ESPIRITU"
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The truth about an animal is far more exciting and altogether more beautiful than all the myths woven about it.

Konrad Lorenz

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Índice

Resumen

La selección sexual es responsable de la evolución de características exageradas y conspicuas. Estas características pueden ser señales honestas de desempeño, calidad genética o condición del individuo, si su producción depende o comparte vías fisiológicas con características vitales, o bien generan compromisos entre ellas y atributos asociados a la supervivencia. Así, estas señales sexuales se usan para evaluar la calidad de posibles parejas, o en la competencia intraespecífica para estimar la capacidad de retención de los recursos del oponente. En la lagartija de collar de Dickerson, *Crotaphytus dickersonae*, los machos exhiben un azul fuerte sobre todo el cuerpo, un collar de dos franjas negras y una blanca, y puntos blancos en el dorso. En este trabajo estudié el papel de la coloración llamativa de los machos en la selección sexual.

En un estudio correlativo evalué si los diferentes parches de color señalan desempeño relacionado con la capacidad de retención de los recursos y estatus inmunológico de los machos. Además evalué si existen compromisos entre la expresión de la coloración sexual y la respuesta inmune, mediados por los niveles de testosterona o por la temperatura corporal. Posteriormente evalué si las hembras en su elección de pareja usan la coloración como señal de calidad del macho y si los machos la usan como señal de la capacidad de retención de los recursos cuando se enfrente a otros machos. Por otra parte, la coloración no necesariamente es estática. Evalué experimentalmente si la coloración es dinámica y si depende de la temperatura. Además investigué experimentalmente si a temperaturas extremas hay un compromiso entre mantener el color (una señal sexual dinámica) y la respuesta inmune.

Encontré que los machos de color azul más fuerte, con collar más grande y obscuro tenían mejor desempeño; corrían más rápido y tenían mayor fuerza de mordida. También machos con collar grande y obscuro tenían mejor estatus inmunológico con mayor inflamación como respuesta a un reto inmunológico y tenían menos parásitos. No encontré evidencia de que el nivel de testosterona en sangre afectara la coloración, el desempeño, o la respuesta inmune. Sin embargo,

la temperatura corporal parece jugar un papel importante en la coloración y la fisiología de estas lagartijas. Los machos con temperaturas corporales más altas fueron de un azul más fuerte, tuvieron un collar más pequeño y menos obscuro. Además, la temperatura corporal afectó positivamente la respuesta inmune.

Los resultados de un estudio experimental sugieren que las hembras usan la coloración de los machos como señal en la elección de pareja. Las hembras se acercan más y pasan más tiempo cerca de machos con un collar negro menos obscuro. Sin embargo ellas desplegaron más hacía machos de un collar negro más obscuro. Cuando el área del collar fue manipulada y la hembra se enfrentó simultáneamente a un macho con collar disminuido, uno con collar aumentado y uno sin manipular el área del collar, las hembras discriminaron en contra los machos con collar aumentado. Las hembras se acercaron con mayor frecuencia y permanecieron más tiempo junto a machos que realizaron más despliegues, pero cuando estos machos tenían un collar aumentado se acercaron menos. Adicionalmente, las hembras se acercaron con mayor frecuencia a machos más azules. Las hembras parecen preferir a machos con un color azul más intenso, pero también con collares menos grandes y menos obscuros, lo que indica que no evaluaron la capacidad de retención de los recursos y el estatus inmune de los machos. Sin embargo, un color azul más intenso, un collar negro pequeño y luminoso señala una temperatura corporal alta, y las hembras podrían preferir a estos machos porque las temperaturas corporales altas pueden aumentar su desempeño físico y fisiológico.

En los experimentos de competencia entre machos ninguno de los parches de color predecía al ganador. No obstante, los machos quienes fueron dominantes en los ensayos, fueron más agresivos hacía machos más azules. También encontré que machos quienes fueron subordinados en los ensayos, tuvieron más conductas sumisas en presencia de machos con un collar más pequeño, lo que va en contra de lo esperado si un collar grande indica mayor capacidad de retención de los recursos. De forma interesante, observé en algunos ensayos que los machos subordinados cambiaron de color azul a café.

En otro estudio experimental, observé que el color azul era menos intenso a temperaturas bajas o altas comparado a temperaturas intermedias. De forma similar, la respuesta inmune bajó a temperaturas extremas, por lo que el color azul puede ser una señal dinámica de la respuesta inmune. Únicamente los machos pequeños tuvieron un compromiso entre color y respuesta inmune a temperaturas intermedias y altas. Esto sugiere que la coloración sexual es costosa y puede ser favorable para machos jóvenes invertir en la supervivencia en lugar de la reproducción.

Mis resultados indican que la coloración de los machos afecta el cortejo de las hembras y la agresión recibida durante encuentros con otros machos. La coloración señala una capacidad de retención de los recursos y la respuesta inmune de los machos, y tal vez aún más importante, su control de la temperatura corporal. Adicionalmente, la coloración azul es una señal dinámica, afectada por la temperatura y la dominancia. Los compromisos entre esta coloración sexual y la respuesta inmune indican que es costoso desplegar la coloración sexual. Lo anterior sugiere que la coloración de los machos de la lagartija de collar de Dickerson juega un papel importante en la selección sexual y enfatiza la importancia de la temperatura corporal en las animales ectotermos.

Palabras claves: señales honestas, coloración, temperatura, respuesta inmune, elección femenina, competencia entre machos, lagartija

Abstract

Sexual selection is responsible for the evolution of exaggerated and conspicuous traits. These traits can be honest signals of whole-body performance, genetic quality or condition of the individual, when the production of the trait depends on or shares physiological pathways with vital traits, or generate trade-offs between the signal and survival traits. These sexual signals are used in mate choice in which quality of possible mates is evaluated, or in intra-sexual competition to estimate the resource-holding power of opponents. In Dickerson's collared lizard, *Crotaphytus dickersonae*, males exhibit a deep cobalt blue colour over the entire body, a black and white collar, and white spots on the dorsum. Here, I studied the function that the striking colouration of the males has in sexual selection.

In a correlational study I evaluated if the different colour patches of the males signal their performance related with resource-holding power and immune status. Also, I evaluated the existence of trade-offs between expressing the sexual colouration and immune response, mediated by the levels of testosterone and body temperature. Subsequently, I evaluated whether females use colouration as a signal of male quality when choosing a mate, and if males use colouration as a signal of resource-holding power when they encounter a male. On the other hand, colouration may not be static. I evaluated experimentally whether colour is dynamic and if it depends on temperature. Furthermore, I investigated experimentally if extreme temperatures generate a trade-off between maintaining colouration (a dynamic signal) and immune response.

Results show that males with a more intense blue colour and those that had a bigger and darker black collar had better whole-body performance; they run faster and had higher bite force. Also, males with bigger and darker collars had better immune status with stronger inflammation response to an immunological challenge and had fewer parasites. I did not find evidence that testosterone levels in the blood affected colouration, performance or immune response. Body temperature, however, played an important role in colouration and physiology of these lizards. Males with higher body temperatures were bluer and had a black

collar that was smaller and lighter. Furthermore, body temperature affected positively the immune response.

Results of an experimental study suggest that females use male colouration as a signal in mate choice. Females approached more and stayed longer near males with a lighter black collar. However, they displayed more to males with a darker black collar. When collar size was manipulated and females encountered simultaneously a male with increased, decreased and non-manipulated collar area, females discriminated against males with a big collar. Females approached and stayed more time near males with high display frequency, but not when these males had collars that were increased experimentally. Furthermore, females approached bluer males more frequently. Females appear to prefer bluer males, but with smaller and lighter collars, indicating that they do not evaluate resource-holding power or immune status. However, a more intense blue colour, and a smaller and lighter black collar signal high body temperatures, and females may prefer these males because their high body temperatures can increase physical and physiological performance.

In the male-competition tests none of the colour traits predicted the winner. However, males who were dominant in the trials were more aggressive to bluer males. Also, I found that males who were subordinate in the trials made more subordinate displays in the presence of a male with a small collar, which was unexpected as a bigger collar signals higher resource-holding power. Interestingly I observed in some trials that subordinate males changed colour from blue to brown.

In another experimental study, I observed that males were less blue at low and high temperatures compared to average temperatures. Also the immune response was lower at extreme temperatures. Therefore blue colour may be a dynamic signal of immune response. Small males showed a trade-off between colour and immune response at average and high temperatures. This suggests that sexual colouration is costly to display and young males may invest in survival rather than reproduction.

The results indicate that male colouration affects courtship behaviour by the females and received aggression during male encounters. Male colouration signals

resource-holding power, immune response, and perhaps more importantly body temperature. Furthermore, the blue colour is a dynamic signal, influenced by temperature and male dominance. Trade-offs between this sexual colour trait and the immune response indicate that this sexual colour may be costly to display. Hence, this study indicates that male colouration of Dickerson's collared lizards plays an important part in sexual selection and emphasises the importance of body temperature in ectotherm animals.

Keywords: honest signals, colouration, temperature, immune response, female mate choice, male competition, lizard

Capítulo 1.

Introducción

Capítulo 1: Introducción

La selección sexual resulta en el éxito reproductivo diferencial entre individuos de la misma especie; algunos individuos producen más crías o crías de mejor calidad (que con más frecuencia logran sobrevivir a edad adulta y reproducirse) que otros (Darwin 1859, 1871). Para maximizar el éxito reproductivo los individuos tratan de excluir competidores del mismo sexo, a lo que se llama selección intra-sexual, y/o atraer a los del sexo opuesto, llamado selección inter-sexual (Andersson 1994). Más recientemente se ha reconocido al conflicto sexual como un tercer mecanismo en la selección sexual (Holland y Rice 1998). El conflicto sexual puede ocurrir cuando los intereses del macho y la hembra no coinciden y puede resultar en seducción antagónica y manipulación, en la que el macho trata de persuadir a las hembras para que se apareen (únicamente) con él, mientras las hembras deben de tratar de resistir las seducciones y manipulaciones de los machos (Ryan 1990, Holland y Rice 1998, Cordero y Eberhard 2003, Parker 2006). Aunque existe la competencia entre hembras y la elección masculina, para simplificar a continuación nos referimos a competencia entre machos y elección de pareja por parte de las hembras.

Para los machos, los beneficios en adecuación a través de cualquiera de los mecanismos por los que opera la selección sexual son muy claros: un aumento en el número de crías producidas. Por su parte, las hembras, al ser selectivas en su elección de pareja, pueden obtener beneficios directos, cuando eligen como pareja machos que defienden un territorio de alta calidad (Emlen y Oring 1977), que invierten en el cuidado parental (Trivers 1972), o machos saludables, evitando así el contagio de enfermedades (Kirkpatrick y Ryan 1991), y/o beneficios indirectos, cuando los machos preferidos, por ejemplo aquellos que despliegan colores llamativos o estructuras elaboradas, heredan también a sus hijos buenos genes que incrementan su viabilidad (Zahavi 1975; Hamilton y Zuk 1982; Kodric-Brown y Brown 1984; Grafen 1990), o cuando los hijos heredan de los padres características que los hacen sexualmente atractivos en la etapa de adultos (Fisher 1930). En consecuencia, la selección sexual es responsable de la evolución de características ligadas a la selección intra e inter-sexual y al conflicto sexual, y en general a las estrategias reproductivas de organismos con reproducción sexual.

En poblaciones silvestres, las hembras receptivas (o sus gametos) son con frecuencia un recurso limitado, por lo cual los machos compiten para obtener un mayor número de copulas. Para conseguir el mayor número de parejas, los machos tratan de llegar antes que las hembras al sitio de reproducción (Morbey y Ydenberg 2001), y en especies territoriales establecen territorios que defienden activamente (Stamps 1994, López-Sepulcre y Kokko 2005). En otras especies, además del territorio los machos pueden defender recursos que pueden ser de interés para la hembra, o bien las hembras pueden ser el recurso que defienden (Stamps 1977). En algunas especies territoriales, hay más de una estrategia en la población y algunos machos pueden no defender un territorio y tratar de robar cópulas dentro del territorio de otro macho. Estos machos satélites incluso pueden tener una morfología similar a la de las hembras para engañar a los machos dominantes y pasar desapercibidos (Sinervo y Lively 1996, Zamudio y Sinervo 2000, Neff y Svensson 2013). Otra estrategia común de los machos es establecer una jerarquía de dominancia, sin necesidad de territorios, a través de conductas y/o características, como parches de color (ver mas adelante) o estructuras elaboradas, que indican su condición o capacidad para pelear, y se ha reportado que machos dominantes copulan más frecuentemente (revisión en Ellis 1995, Wroblewski et al. 2009). Las estrategias antes mencionadas no son excluyentes y varias de ellas pueden observarse en la misma especie (e.g. Sinervo 2000), o incluso un macho puede cambiar de estrategia dependiendo de las circunstancias y/o su condición (Gross 1996).

Características sexuales secundarias llamativas: Señales honestas

La selección sexual favorece el desarrollo de características sexuales secundarias. Estas características incluyen estructuras que sirven para el combate o la intimidación de otros miembros del mismo sexo, por lo que se han llamado armamentos. O bien son estructuras, conductas y/o coloraciones muy llamativas y elaboradas que pueden tener la función de atraer a miembros del sexo opuesto (en estos casos se les llama ornamentos), o señalar las habilidades competitivas a posibles rivales (señal de estatus; Berglund et al. 1996). Los ornamentos y señales de estatus probablemente son costosos de producir y mantener y podrían disminuir la supervivencia del individuo que los despliega (Darwin 1871), de tal manera que es más factible que el carácter sea una estrategia evolutivamente estable cuando se trata de una señal honesta (Zahavi 1975). Las señales honestas son características que informan a otros individuos de manera confiable la calidad (i.e. su desempeño físico y fisiológico en características relacionadas a la adecuación; Lailvaux y Kasumovic 2011), o condición (i.e. la capacidad de mantener un desempeño óptimo de los procesos celulares vitales; Hill 2011) del individuo que la despliega. Los conceptos de condición y calidad de los individuos son clave para entender como la selección sexual favorece la evolución y el mantenimiento de las señales. Sin embargo, a menudo no son fáciles de medir en estudios de campo por lo que se han usado algunas características (“proxies”) como indicadores de calidad y condición. Por ejemplo, se han usado como indicador de calidad medidas de desempeño importantes en las interacciones de competencia intra-sexual, como es la fuerza de mordida, la resistencia y velocidad para correr, o bien se han usado algunos componentes de la respuesta inmune para estimar la resistencia ante patógenos (ver Roberts et al. 2004, Irschick et al. 2008). Mientras como indicadores de condición generalmente se considera el peso o el índice corporal (Schulte-Hostedde et al. 2005). Sin embargo, en la actualidad se reconoce que entre variables tradicionalmente usadas como indicadores de condición y calidad pueden existir compromisos y estos compromisos pueden variar en función de las condiciones ecológicas, sociales, o de la misma condición del individuo. Consecuentemente, para evaluar la condición o calidad de un individuo es necesario considerar simultáneamente un conjunto de características y una aproximación de historias de vida (Wilson y Nussey 2009, Lailvaux y Kasumovic 2011).

Las señales llamativas han recibido mucha atención. Aunque es posible que una señal se mantenga debido a que las hembras prefieren esta característica y por lo tanto las hembras que se aparean con los machos preferidos tendrán hijos que a su vez son “atractivos” (Fisher 1930, Weatherhead y Robertson 1979, Pomiankowski y Iwasa 1993), se piensa que es más factible que una señal evolucione cuando se trata de una señal honesta (Fisher 1958). De acuerdo al principio del hándicap, las características llamativas son honestas porque sólo los individuos de alta calidad pueden sobrevivir con este hándicap (Zahavi 1975, 1977). Por otro lado, se ha considerado que la resistencia a parásitos y enfermedades puede ser una de las características más importantes para la supervivencia de los animales (Lochmiller y Deerenberg 2000). Así, se espera que el despliegue de señales que indican la resistencia a las infecciones por parásitos sea favorecida por la selección sexual, a esta idea se le conoce como la hipótesis de la inmunocompetencia (Hamilton y Zuk 1982). Las señales podrían ser señales honestas de la inmunocompetencia por ejemplo cuando la señal y la función inmune comparten vías fisiológicas o hay compromisos fisiológicos entre ellas (Hill 2011). De acuerdo con las ideas anteriores, Folstad y Karter (1992) sugieren en su Hipótesis del Hándicap de la Inmunocompetencia que la testosterona al ser la hormona sexual principal de los machos, está involucrada en el desarrollo de caracteres masculinos y simultáneamente puede tener efectos inmunosupresores. Por lo anterior, las señales sexuales que dependen de testosterona podrían considerarse como señales honestas de la condición de un individuo debido a que sólo animales resistentes a las enfermedades pueden pagar el costo inmunosupresor de mantener altos niveles de esta hormona.

Colores llamativos en Lagartijas

En las lagartijas, un gran número de especies presentan coloraciones llamativas, aunque con más frecuencia es el macho que presenta un fenotipo más colorido (Cooper y Greenberg 1992). A pesar de que los colores conspicuos pueden aumentar la depredación (ej. Martín y López 1999), de acuerdo a la selección sexual los machos con coloración más conspicua tendrán una adecuación más alta, si las hembras los prefieren o el color señala el estatus de dominancia (Anderson 1994, Olsson 1994). Por su lado, si el color es una señal honesta del desempeño del macho, la calidad de sus genes, su inversión parental o su atractivo sexual, las hembras que se aparean con machos de colores brillantes aumentarían su adecuación (Anderson 1994). Pelear es una conducta costosa: mientras pelean, los animales pierden energía, se vuelven más conspicuo a depredadores y al mismo tiempo están menos alerta, además de incurrir en el riesgo de ser herido (Marler y Moore 1988, Olsson 1994, Martín y López 2001). Los colores que señalan la capacidad de pelear o el estatus de un animal reducen los costos de la pelea debido a que los competidores pueden estimar sus oportunidades para ganar y sólo pelear cuando sus posibilidades de ganar son altas (Maynard Smith y Price 1974, Olsson 1994). Por otro lado, los colores conspicuos pueden ser un hándicap, y por lo tanto una señal honesta del desempeño del organismo, debido a que un animal colorido también puede ser más llamativo para los depredadores (Martín y Lopéz 1999, Stuart-Fox y Moussalli 2008, Macedonia *et al.* 2009), y más visible para sus presas (Baird 2008). También los colores pueden ser costosos de producir o mantener por los pigmentos que requieren (Lozano 1994, McGraw 2003), o bien porque exigen la presencia de testosterona (Folstad y Karter 1992).

Testosterona

En lagartijas, se ha encontrado que el nivel de testosterona en la sangre y el color que despliegan los machos están relacionados (Hews et al. 1994, Sinervo *et al*. 2000, Cox *et al*. 2005), además la testosterona aumenta el tamaño de los parches de color (Salvador et al. 1996) y la intensidad del color (Cox *et al*. 2008). Conjuntamente, se han encontrado relaciones positivas entre el nivel de testosterona y la talla de los machos (Husak *et al*. 2007, John-Alder *et al*. 2009), su desempeño en términos de velocidad (Klukowski *et al*. 1998, Klukowski y Nelson 1998), resistencia (Sinervo *et al*. 2000, John-Alder *et al*. 2009) y fuerza de mordida (Husak *et al*. 2007), los cuales son importantes en la dominancia de los machos y afectan su éxito reproductivo (Robson y Miles 2000, Olsson *et al*. 2002, Lappin y Husak 2005, Peterson y Husak 2006, Lailveax y Irschick 2007, John-Alder *et al*. 2009). Sin embargo, tener altos niveles de testosterona en la sangre disminuye la respuesta inmune celular (Oppliger *et al*. 2004, Belliure et *al*. 2004, Berger *et al*. 2005) y se relaciona con un aumento en el número de ectoparásitos (Salvador *et al*. 1996, Klukowski y Nelson 2001, John-Alder *et al*. 2009). Por lo tanto, es probable que los machos se enfrenten a un compromiso, mediado por la testosterona, entre un buen desempeño, un color atractivo y mantener una buena capacidad de respuesta inmune como propone la hipótesis de inmunocompetencia (Folstad y Karter 1992).

Temperatura

En los animales ectotermos, la temperatura corporal afecta el desempeño motriz (Bennett 1980, Huey 1982), debido a que la contracción y relajación de los músculos dependen de la temperatura (Bennett 1985). Consecuentemente la temperatura afecta desempeños como la velocidad con que corren los animales o su resistencia, pero no la fuerza de mordida que no depende tanto de las contracciones musculares (Bennett et al. 1985, Herrell et al. 2007, Segall et al. 2013). Además, por la cinética enzimática las temperaturas más altas aumentan la eficiencia de las reacciones fisiológicas, resultando en un mejor desempeño fisiológico, hasta un límite en el cual, por deterioro de las enzimas, se baja el nivel de desempeño (Huey 1982, Angilletta 2009). En ectotermos, uno de los componentes fisiológicos que se ve afectado por la temperatura es el sistema inmune (Sypek et al. 1984, Le Morvan et al. 1998, Zimmerman et al. 2010, Merchant et al. 2014). Por ejemplo, en la lagartija *Hemidactylus flaviridis* la capacidad de fagocitosis es inferior a bajas y altas temperaturas de 7, 15 y 37ºC, comparada con temperaturas intermedias de 25ºC (Mondal y Rai 2001).

La temperatura también puede afectar la coloración en ectotermos. En algunas especies de lagartijas que habitan áreas con temperaturas bajas se ha sugerido que el hecho de que son con frecuencia más obscuras, les ayuda a calentarse más rápido (Clusella-Trussel et al. 2007). Por otra parte, hay animales que pueden obscurecerse en tiempos relativamente cortos (menos de una hora) como respuesta a temperaturas bajas (Sherbrooke 1997). El obscurecimiento rápido de la piel resulta de la dispersión de los melanosomas (pigmentos que causan colores obscuros), especialmente cuando los melanosomas se dispersan por las dendritas de melanoforos que van sobre las capas de pigmentos superiores (Taylor y Hadley 1970, Grether et al. 2004). Además, la temperatura puede ocasionar cambios en la dispersión y congregación de iridóforos (pequeños plaquetas que reflejen la luz; Morrison et al. 1996). A temperaturas altas, hay menos distancia entre los iridóforos lo que causa colores más saturados en el azul y/o UV (Morrison et al. 1996). El azul y UV frecuentemente son colores usados como señales sexuales en lagartijas (Cooper y Greenberg 1992). Por ejemplo en la lagartija *Lacerta viridis,* las hembras prefieren machos que reflejen más en el UV en su parche gular (Bajer et al. 2010), y la saturación del UV es más alta en machos que pasan más tiempo a temperaturas altas (Bajar et al. 2012). En la lagartija *Sceloporus undulatus* los parches de color de la garganta y ventral señalan dominancia en los machos y cambian de color con la temperatura, de verde cuando están fríos, a azul cuando se han calentados (Langkilde y Boronow 2012). Por consiguiente los colores de un individuo no necesariamente son estáticos (i.e. no se mantienen igual por largos periodos de tiempo), sino pueden ser dinámicos. Se han encontrado que los colores pueden cambiar en sincronía con la condición corporal (e.g. Velando et al. 2006, Pérez-Rodríguez y Viñuela 2008), el estatus social (Setchell y Dixson 2001, Karubian et al. 2011) o la salud (Faivre et al. 2003, Rosenthal et al. 2012). Las señales dinámicas pueden ser útiles para indicar la adaptación del animal a los cambios en condiciones ambientales (Bro-Jørgensen 2010) y permite una evaluación continua de la pareja que puede ser especialmente importante en especies monógamas con cuidado parental (Torres y Velando 2003). Pero también puede ilustrar beneficios directos, cuando señala si el individuo está combatiendo una enfermedad posiblemente contagiosa (Bro-Jørgensen 2010) o cuando señala dominancia y por lo tanto control sobre recursos y protección que puede ofrecer a la pareja (Qvarnström y Forsgren 1998). También, cuando señala estatus social puede reducir la agresión en interacciones con co-específicos del mismo sexo (Karubian et al. 2011). Por otro lado, el animal podría reducir la agresión que vaya a recibir y los costos de mantenimiento de la señal al sólo desplegar señales de dominancia cuando tiene buenas oportunidades de ser dominante (Karubian et al. 2011).

Señales múltiples

Muchos animales despliegan más de una señal simultáneamente, así como los parches de color y la conducta, pero también diferentes parches o diferentes aspectos del mismo parche de color (como saturación, color y tamaño) pueden funcionar como señales múltiples (Grether et al. 2004). Las señales múltiples pueden indicar diferentes cualidades de los machos (Møller y Pomiankowski 1993, Candolin 2003, Grether et al. 2004). Por ejemplo, en la lagartija *Lacerta schreiberi* el azul y UV en la cabeza señalan dominancia, pero el parche de color amarillo ventral se relaciona con la respuesta inmune (Martín y López 2009). Sin embargo, según la hipótesis de redundancia señales múltiples pueden indicar también las mismas características. Esto permite una evaluación más fácil del macho por los individuos conespecíficos y hará más difícil que se generen engaños (Møller y Pomiankowski 1993, Candolin 2003, Számadó 2011). En lagartijas se ha demostrado que las hembras evalúan varias señales simultáneamente en los machos cuando escogen pareja (Lancaster et al. 2009, Swierk et al. 2012). Por ejemplo, las hembras de *Uta stansburiana* evalúan la coloración de la garganta y dorso de los machos simultáneamente, ya que la garganta indica estrategia reproductiva, mientras la coloración dorsal se relaciona con la supervivencia (Lancaster et al. 2009). Por lo tanto, los machos pueden desplegar múltiples parches de color para indicar varios aspectos de su condición, desempeño, estatus de dominancia o buenos genes, o bien puede aumentar la eficiencia de la comunicación al señalar la misma información con varios parches de color.

La Lagartija de Collar

Mi sujeto de estudio fue la lagartija de collar *Crotaphytus dickersonae*. La especie presenta un dimorfismo sexual pronunciado en talla y color (Fig. 1). Los machos son 14% más grandes y 63% más pesados que las hembras (Plasman *et al*. 2007). Esta lagartija tiene un patrón de coloración complejo. Los dos sexos tienen puntos blancos en el dorso y un collar de tres bandas, dos negras divididas por

Figura 1. Lagartija de collar de Dickerson *Crotaphytus dickersonae*. El macho al frente y la hembra atrás. Existe un marcado dimorfismo en talla (los machos son 14% más grandes y 63% más pesados que las hembras) y color. La hembra despliega rayas naranjas que indican su receptividad sexual. Foto por William Wells

una banda blanca. Las hembras son pardas con patas amarillas, mientras que los machos son de color azul fuerte (McGuire 1996, Macedonia et al. 2009). Esta lagartija tiene una visión de color muy buena (Macedonia et al. 2009) y es altamente probable que pueden distinguir entre variaciones pequeños en color. Aunque pueden percibir el UV, el dorso y el collar negro refleje muy poco en este rango (Macedonia et al. 2009). Esta lagartija vive en el hábitat desértico del estado de Sonora en México, donde únicamente se encuentra en la costa entre Bahía de Kino y Puerto Libertad, y en Isla Tiburón (McGuire 1996). En este hábitat abierto con poca vegetación la coloración de los machos los hace muy conspicuos. Además los machos frecuentemente realizan despliegues en lugares altos, lo que aumenta su detectabilidad. Los machos son altamente territoriales, con territorios que se sobreponen con los rangos hogareños de varias hembras (McGuire 1996).

Los machos realizan despliegues conductuales que enfatizan su coloración a conespecíficos de ambos sexos. Entre estos despliegues está la conducta llamada “full show” (en inglés), que se caracteriza por exposición de las partes corporales laterales hacia el co-especifico, y extensiones del área gular enfatizando así el collar en este especie. En *Crotaphytus collaris* la frecuencia de estos despliegues se relaciona con la intensidad de agresión y cortejo (Baird et al. 1997, Husak 2004, Baird 2013). En *C. dickersonae* se observan los mismos despliegues (obs. pers.).

Objetivos

El objetivo de este estudio es investigar el papel de diferentes características de la coloración de los machos de la lagartija de collar *Crotaphytus dickersonae* como señales sexuales. En primer lugar, en un estudio correlativo evalué si la coloración (1) es una señal honesta del desempeño y respuesta inmune del macho, (2) si depende de los niveles de testosterona y la temperatura corporal y (3) si hay un compromiso entre mantener una coloración llamativa y la función inmune (capítulo 1). Para entender la posible función de la coloración en las interacciones con co-específicos en el contexto de selección sexual evalué si la coloración es usada como señal en la elección femenina precopulatoria (capítulo 2) y la competencia entre machos (capítulo 3). Posteriormente evalué experimentalmente si la coloración de los machos es dinámica y depende de la temperatura, y si hay un compromiso entre mantener el color (una señal sexual dinámica) y la respuesta inmune (capítulo 4).

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Capítulo 2.

Multiple colour traits signal performance and immune response in the Dickerson's collared lizard *Crotaphytus dickersonae*

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ORIGINAL PAPER

Multiple colour traits signal performance and immune response in the Dickerson's collared lizard *Crotaphytus dickersonae*

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Abstract Conspicuous male coloration is often an honest signal of individual quality. In Dickerson's collared lizard (*Crotaphytus dickersonae*), males are striking blue, have a black and white collar and have white spots on their bodies. In this study, we investigated whether this extravagant colour pattern is an indicator of male performance in variables related to resource-holding power (bite force, running speed and endurance) and immune condition (cellular response and ectoparasite load). Also, we assessed direct and indirect effects of testosterone levels and body temperature on colour traits. We found that males with bluer skin, and darker and bigger black collars performed better in variables related to resource-holding power. Also, the black colour of the collar was related to immune condition. Blue chroma was positively related to temperature, yet males with bigger and darker black collars had lower body temperatures. Testosterone had minor effects on immune response and colour traits. Our results suggest that multiple colour traits in the Dickerson's collared lizard may honestly indicate physical and physiological performance.

Keywords Honest signal · Coloration · Temperature · Testosterone · Immune condition · Resource-holding power

Introduction

According to sexual selection theory, in many species, conspicuous coloured males have evolved due to sexual selection (Anderson 1994). More colourful males are expected to have increased fitness, either because colour traits are favoured by mate choice or colour functions as a signal of status in intrasexual competition. Communication via colour traits will be evolutionarily stable when the colour reflects performance, condition or genetic quality of the animal, and colour can thus be considered an honest signal (Guilford and Stamp Dawkins 1991). In theory, honest signalling is maintained when the potential costs of cheating are high (Számadó 2011) or when high- and low-quality individuals differ in some components of their physiological state or condition that influence their health, performance or ability to acquire resources, resulting in constraints or higher costs for lower quality individuals to produce and maintain a high intensity signal (Zahavi 1975, 1977; Hamilton and Zuk 1982; Maynard Smith 1991; Johnstone 1995). For example, conspicuous coloration can be an honest signal when only individuals in good condition can pay the costs of increased visibility to predators (Endler 1992; Martín and López 2001) or prey (Baird 2008). Additionally, development and maintenance of pigment-dependent colour traits might be costly when the pigments involved are difficult to obtain or are required for other essential functions such as the immune function (Lozano 1994). However, there is no need of costs of production for colour traits to be honest indicators of condition. Colour traits might be linked to condition when regulatory agents that stimulate

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colour expression (e.g. testosterone) simultaneously depress or share pathways with other fundamental physiological processes (Hill 2011).

Hormones have been targeted as a key mechanism controlling reproduction through their sex-specific influence on the expression of morphological and behavioural traits, including the display of secondary sexual characters such as the conspicuous colours of many animals (Kimball 2006). For instance, testosterone stimulates muscle development (Herbst and Bhasin 2004; Huyghe et al. 2009), and therefore whole-organism performance on traits that influence survival and intra-sexual competition and dominance, hence contributing to overall fitness (Ellis 1995; Husak and Fox 2008; Irschick et al. 2008). Accordingly, in lizards, testosterone levels have been found to influence running speed, endurance, and bite force, which are performance traits that influence male-male competition and dominance (Klukowski and Nelson 1998; Klukowski et al. 1998; Olsson et al. 2000; Robson and Miles 2000; Sinervo et al. 2000; Lappin and Husak 2005; Peterson and Husak 2006; Husak et al. 2007; Lailvaux and Irschick 2007; John-Alder et al. 2009). Furthermore, testosterone is often required for the development of male colour traits favoured by sexual selection (Hews et al. 1994; Salvador et al. 1997; Sinervo et al. 2000; Cox et al. 2008; Olsson et al. 2012). However, elevated levels of testosterone can also impose costs, by suppressing immunocompetence and increasing parasitism (Hamilton and Zuk 1982; Folstad and Karter 1992; Roberts et al. 2004), resulting in a decrease of individual fitness (Cox and John-Alder 2007). Particularly, it has been suggested that if testosterone is responsible for the expression of colour traits, and simultaneously has immunosuppressive effects, only individuals in good condition or with resistant genes could afford to display testosterone-dependent colourful traits (Folstad and Karter 1992). Thus, testosterone-dependent colour traits are expected to be honest signals of male competitive ability and individual condition.

In lizards, recent studies report a temperature dependence of structural coloration. Higher body temperatures are related to more reflection in the UV and blue range (Hettyey et al. 2009; Bajer et al. 2012; Langkilde and Boronow 2012). In the Eastern Fence lizards (*Sceloporus undulatus*), the male's throat and ventral colour patches changed from green to blue when the lizards were moved from low to high temperatures (Langkilde and Boronow 2012). Body temperature is very important for ectothermic animals, as it influences their growth, performance, and immune response (Bennett 1980; Huey 1982; Angilletta et al. 2002; Zimmerman et al. 2010). Typically, at optimal temperature, organisms have higher physiological and physical performance, compared to performance at lower or higher temperatures. Furthermore, animals with greater thermoregulation capacity may be active within a larger range of environmental temperatures, will have less microhabitat restriction and increased activity time, resulting in more time to forage, territory defence and mate searching. Hence, particularly in animals that live in habitats with extreme temperatures, colour traits that depend on body temperature might be honest indicators of physiological and physical performance (Bajer et al. 2012; Langkilde and Boronow 2012).

Animal coloration consists often of multiple colour traits that in the context of sexual selection might convey information on the bearer's quality. According to the redundancy hypothesis, different colour traits reflect the same information of individual quality, allowing for a better and faster assessment of potential mates or competitors, reducing the time spent on assessment and the probability of errors, and facilitating the detection of cheaters (Møller and Pomiankowski 1993; Candolin 2003). Alternatively, the multiple messages hypothesis posits that different colours or different components of the same colour trait (e.g. hue, lightness, size or number of patches) are produced through different pathways and can therefore indicate different aspects of individual quality (Møller and Pomiankowski 1993; Candolin 2003; Grether et al. 2004). For example, red, orange and yellow colour patches are typically produced by carotenoid pigments, which are obtained from the diet, and might function as antioxidants and immunostimulants; then, carotenoid-dependent colour traits have been considered as indicators of both nutritional and immune status (Lozano 1994). Brown and black colours are produced by melanins and are frequently related to dominance status (e.g. Jawor and Breitwisch 2003). Melanins are endogenously produced and genes involved in melanogenesis affect other physiological aspects of the individual such as the immune response (Ducrest et al. 2008). Additionally, in ectotherms, melanin-dependent colours have been associated to thermoregulation, shortening the time necessary to heat up and hence increasing the time the animal can perform optimally (Clusella Trullas et al. 2007). On the other hand, structural colours, such as blue and UV, result from the selective reflectance of light by a diversity of structures in the skin (Umbers 2013). For example, in vertebrates, the arrangement of iridophores, a type of chromatophore that contains crystalline structures, may give rise to blue (Morrison et al. 1996; Umbers 2013). Structural colours will thus require precise development; as a result of this, it has been suggested that they might indicate good genes (Shawkey et al. 2003; Umbers 2013). In addition, in ectotherms, structural colours may require an adequate body temperature (Hettyey et al. 2009; Bajer et al. 2012; Langkilde and Boronow 2012). Consequently, by displaying different colour traits, individuals may signal different aspects of their condition, performance or social dominance, or may reinforce through multiple signals the same information about its quality.

The Dickerson's collared lizard *Crotaphytus dickersonae* inhabits the Sonoran desert in Mexico, an open habitat with sparse vegetation cover and air temperatures that can reach over 50 °C. These lizards have a strong sexual dimorphism in size and colour. Males are on average 14 % larger and 63 % heavier than females (Plasman et al. 2007). Females are brownish with yellow legs and tail, while the body of males is deep cobalt blue (McGuire 1996; Macedonia et al. 2009). Both sexes have white spots on the dorsum and a collar of two black bands with a white stripe in the middle. The possible function of male coloration in this species is presently unknown. However, in the closely related species, *Crotaphytus collaris*, females have been found to prefer brighter males (Baird et al. 1997). Furthermore, in *C. collaris* displays such as full show or gular extension, in which the male exhibit its coloration (e.g. Macedonia et al. 2004), can be seen in interactions with both male and female conspecifics and have been related to the intensity of aggression and courtship frequency (Baird et al. 1997; Husak 2004; Baird 2013). Similar display behaviours have also been seen in *Crotaphytus dickersonae* (MP personal observation). Hence, the conspicuous male coloration of the Dickerson's collared lizard might be under sexual selection.

In this study, we evaluated whether male colour traits (the blue colour of the body, the white spots on the dorsum and the collar) of the Dickerson's collared lizard are indicators of performance (bite force, running speed and endurance), immune response and parasite load. Testosterone has been associated to colour displays and performance, and, in lizards, body temperature may modulate colour, performance and physiology. Therefore, we tested for direct and indirect relationships between circulating testosterone levels, body temperature, and colour and performance traits. Finally, to evaluate whether testosterone may function as an immune suppressor, we examined the relationships between testosterone and immune response and parasites load.

Methods

The study was conducted in Bahía de Kino, Sonora, Mexico. Forty eight males were captured between Bahía de Kino and Punta Chueca from April to June 2011, during the breeding season of *C. dickersonae* (McGuire 1996). Within 4 min after capture, males were blood sampled by taking 100 μl of blood with a 1-ml syringe from the caudal vein in the tail. Samples were stored on ice and within 4 h after collection were centrifuged (10,000 rpm during 10 min) to separate the plasmatic fraction from the cellular one. Plasma was stored in liquid nitrogen during fieldwork and then in a deep freezer (−70 °C) until hormone assays were performed. After blood samples were taken, water was given directly in the mouth of each animal to avoid dehydration. Animals were brought in individual cloth bags to a research station in Bahía de Kino where they were kept for the stretch of the tests. Bahía de Kino is within their distribution range and thus allows for natural day-night rhythm and temperatures. Once in the field station and before any other manipulations that might make the parasites to leave the host, all ectoparasites present on the males and in the bags used for transportation were collected. Ectoparasites were counted per host and stored in ethanol (70 %) for later determination to family level.

In captivity, males were individually housed in outdoor cages (60 × 60 × 60 cm) made of wooden frames covered with mesh. Canvas between adjacent cages and in the back of the cages avoided visual contact among males and reduced stress produced by people passing by. Every morning, lizards were provided with food (one or two live crickets depending on their size) and water ad libitum, and cages were misted.

Colour, body temperature and morphological measurements

On the day of capture, three measurements of the background blue on the dorsum and one measurement from each of the two black bands of the collar were taken with a portable spectrophotometer that determines reflectance from 360 to 740 nm at 10-nm intervals (Minolta CM-2600d; Minolta Co. Ltd, Osaka Japan). Although some lizard species can change colour due to the stress of handling (e.g. Greenberg 2002), in the Dickerson's collared lizard no colour changes were observed between capture and colour measurements. Immediately after colour measurements, cloacal temperature, an indicator of body temperature, was measured with a cloacal thermometer (±0.1 °C; Miller and Weber, Inc). Colour and body temperature measurements were completed within 2 to 3 min, and all measurements were taken between 15:00 and 17:00 hours. In ectotherms, body size influences the rate of heat loss, with bigger animals cooling at slower rates than smaller animals (Claussen and Art 1981). To avoid biases from differential heat loss caused by lizard size, before colour and body temperature measurements, lizards were sunbathed for 60 min to allow them to return to their preferred body temperature during the active time of the day. Body temperatures of males after sunbathing did not differ from body temperatures of male lizards measured immediately after capture in the same field site for a different study in a previous year (mean ± SD, present study 37.33 ± 0.175 °C, $n=39$; males from 2005, 37.33 ± 0.178 °C, $n=43$ from Plasman et al. 2007; $t=-0.14$, $P=0.99$). Hence, it is unlikely that our estimates of body temperature might be biased by the size of the individual or the time elapsed between colour and body temperature measurements (roughly 1–2 min).

After colour and temperature measurements, three pictures were taken from the lateral and dorsal sides to estimate the area of the collar and the number and area of the white spots by counting the number of pixels per colour using the histogram option of Adobe Photoshop (version 8.0). Lizards were placed on a flat white surface with a ruler as a scale reference.

Pictures were taken from 40 cm distance with a Nikon Coolpix camera. For each male, the snout-vent length (hereafter SVL; ±1 mm) and the body mass (PESOLA scale±0.1 g) were measured.

From the reflectance colour curves of each lizard, we calculated the blue chroma as the sum of reflectance from 400 to 480/sum of total reflectance of the mean reflectance curve of the dorsum (this index includes reflectance from violets and blue wavelengths; however, for simplicity, we will refer to it as blue chroma; Endler 1990). A lizard with higher blue chroma displays a deeper cobalt blue than those with lower blue chroma, which exhibit a more turquoise colour. Also, the lightness of the black collar bands was measured (sum of total reflectances of the mean reflectance curve of the collar); high lightness indicates a relative light black collar and low lightness a darker black collar.

Male competitive abilities: bite force, running speed and endurance

As a proxy of bite force, we estimated the size of the adductor mandibulae complex (AMC) by making the lizard open its mouth to the maximum and taking a photograph from the front. The photograph was taken after the photographs for colour measurements and included a ruler to allow for scale. The AMC was estimated from the photographs as the distance from the middle of the coronoid bone to the extreme outside of the cheek (Lappin et al. 2006) using ImageJ (version 1.44o). AMC have been found to correlate with bite force in *C. collaris* (Lappin et al. 2006).

To estimate running speed, each male was chased down a racetrack (Husak 2006). The racetrack was made of wood (2.44×0.40×0.40 m) and covered with sandpaper to improve traction. At every 25 cm, a stripe was drawn, which allowed for the estimation of running speed on parts of the racetrack. A dark bag at the end of the track stimulated the animal to target a refuge. The lizard was tested in three continuous runs. The tests were videotaped with a Sony Digital 8 Handicam. From the videos, running speed was estimated, and the faster meter in any of the three runs was taken as maximal running speed.

Endurance was estimated by forcing the lizard to walk on a treadmill (Robson and Miles 2000; Sinervo et al. 2000; Mills et al. 2008). The treadmill consisted of a continuous belt that was moved by hand at 2 km/h. A box made of wood of 50×40×40 cm prevented the lizards to escape. A Plexiglas window in the frontal side of the box gave the impression of an open area and thus a direction in which to walk. The lizard was stimulated to walk by tapping it gently on the flanks and was considered exhausted when it did not respond to three continuous taps (±1 seg; Robson and Miles 2000; Sinervo et al. 2000; Mills et al. 2008). All tests were performed during the hours the lizards were seen active in their cages (from 9:00 to 13:00 and 15:00–17:30 h). Lizards completed the running speed and endurance tests on different consecutive days (second and third day after capture). The order in which a lizard completed these tests was at random, and the order of the tests did not influence performance ($P > 0.18$).

Cell-mediated immune response

After locomotor tests had been completed, to evaluate in vivo local cellular immune response, males were injected subcutaneously in the palm of the right front foot 37 μl of a solution of 5 mg of Phytohemagglutinin-P lectin of the red kidney bean *Phaseolus vulgaris* (PHA; Sigma L8754, St Louis, USA) in 1 ml of phosphate buffered saline solution (PBS) (Belliure et al. 2004; Berger et al. 2005; Mills et al. 2008). The point of injection was marked with an indelible marker and swelling at the point of injection was estimated as the difference between thickness of the manus before and 12, 18 and 24 h after injection, as measured with a digital micrometer (±0.001 mm; Mitutoyo). More swelling is related with a greater infiltration and proliferation of macrophages and lymphocyte; hence, local inflammatory response to the PHA mitogen (hereafter PHA response) has been used as an index of the strength of the cell-mediated immune response (e.g. Belliure et al. 2004; Berger et al. 2005). As inflammation was maximal at 12 h, these measurements were used for the analyses. Because after locomotor tests males were included in a mate selection test (M. Plasman and R. Torres in preparation), cell-mediated immune response was measured on average 7 days after capture.

Testosterone determination

Steroid extractions from plasma were performed by a double ether extraction. Briefly, the amount of plasma obtained was measured with a pipette (±1 μl), and 1 ml of anhydrous ether [$((C_2H_5)_2O)$; JT Baker, Center Valley, USA, code 9244-02) was added. Samples were then set for 30 min in a vortex and centrifuged for 5 min at 2000 rpm. Liquid and solid parts were separated. The ether was evaporated leaving the hormone behind. To the solid part, 1 ml of ether was added and the protocol repeated. To obtain a solution with the original dilution, the hormone extract was dissolved in an equal amount of EIA buffer (Cayman Chem., Ann Arbor, USA, code 400060), as has been the original plasma sample. The solution was shaken to dissolve and to recover all hormones from the tube walls. Levels of testosterone were then determined with a testosterone ELISA kit (Cayman Chem., Ann Arbor, USA, code 582701). The kit uses mouse antibodies and has a lower detection limit of 6 pg/ml and an IC_{50} of 32 pg/ml. Samples were initially diluted three times; however, to obtain values within the detection range of the ELISA, 86 % of the samples had to be diluted another ten times resulting in a final dilution of 30 times. Samples were analysed in duplicate with respect to a standard curve, and the average of duplicates was used for the analyses. Intra-assay coefficient of variation was 6.2 % as measured from duplicates within plates, while inter-assay coefficient of variation was 5.9 % as calculated from a pooled sample included throughout the plates. Testosterone level was not correlated to the time elapsed from capture to blood sampling ($P=0.55$) or to capture date ($P=0.26$); therefore, all samples were included in the analyses.

Statistical analyses

Because physiological variables and variables that estimate male competitive abilities are probably correlated with each other, we used a path analytical approach to evaluate simultaneously the potential covariation between independent variables and their direct and indirect effects on male colour traits. Path analysis provides a method in a multiple regression framework for structuring hypothetical causal relationships between multiple traits (Wright 1921). For the path analysis, we first defined alternative models that represent different biological hypothesis of whether male colour may indicate performance and immune condition (immunocompetence and parasite load), and the potential effects of plasma testosterone level (hereafter T-level) and body temperature on colour displayed. For example, to evaluate the role of T-level as a mediator of male colour, we analysed direct effects of T-level on the colour, and possible indirect effects through the relationship of T-level with PHA response and parasite load, as testosterone is hypothesised to be an immune suppressor (e.g. Folstad and Karter 1992). In a path analysis, the strength of a direct effect is given as the direct path coefficient. This indicates the strength of the independent variable over the dependent variable, corrected for the effects of other variables included in the model. The effect of a variable on the response variable through its influence on other variables is called indirect path coefficient and is calculated by multiplying the path coefficients on the route between variables. The sum of direct and indirect path coefficients is the total path coefficient (Bentler 1989).

The different colour patches were analysed in separate path analyses. Initial models included SVL, body temperature, T-level, AMC, endurance, running speed, PHA response and parasite load. Body mass and SVL were strongly correlated ($R=0.88$, $P<0.01$, $n=48$); hence, only SVL was included in the models. T-level and endurance were log-transformed before analyses. To evaluate the potential immune suppressor effect of T-level, we assessed a model that tested the effects of T-level on PHA response and parasite load, allowing simultaneously for the effects of body temperature and SVL. Subsequently, we fixed the relationships with T-level to zero and compared models with a Wald test (Bentler 1989).

From the initial hypothetical path models, the analysis evaluates the degree of fit between the expected covariance structures and the observed data. The goodness-of-fit of the model was evaluated with a χ^2. A significant χ^2 ($P<0.05$) indicates that the data differs from the expected covariances and the model is rejected. Due to missing data during field work, our sample size for the analyses varied between models from 32 to 48 males. Hence, because sample size was small, we also used the comparative fit index (CFI) and the standardised root mean square residual (SRMR) to reduce type I and II errors (Hu and Bentler 1999). CFI compares the model against a baseline model in which all variables are considered independent. CFI depends on the correlations between variables and gives a higher value for higher correlations, and a CFI value of 0.96 or higher indicates that the model is in accordance to the data (Bentler 1989). SRMR calculates standardised residuals; therefore, a smaller value is better and models with SRMR smaller than 0.09 are considered a good fit to the data (Hu and Bentler 1999). Further, we used Akaike's Information Criterion (AIC) to select the best model. AIC compares between models and optimises the trade-off between complexity and variance. AIC values indicate the amount of information lost, assuming that the data was generated in the process suggested by the model, and a lower value is better (Anderson 2008). Path analyses were performed in R-program (version 2.15.2) package Lavaan (Rosseel 2012).

Results

Blue chroma

Path analysis suggests that in the Dickerson collared lizard, blue chroma is related to indicators of performance, but not to parasite load or PHA response (Fig. 1). Males with bluer body

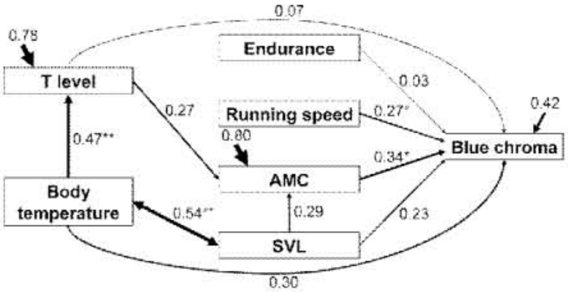

Fig. 1 Path model illustrating effects on blue chroma of performance measures (endurance, running speed and AMC), SVL, T-level and body temperature (model's $\chi^2=13.22$, $df=11$, $P=0.28$, AIC=291.46). Thickness of the *arrows* indicates strength of the relation. *Double-headed arrows* indicate a covariance. *Single-headed arrows* indicate an effect of the variable at the base of the *arrow* over the variable at the head of the *arrow*. *Single-headed arrows* without a *box* at the base of the *arrow* indicate effects of unmeasured factors. Standardised estimate coefficients are given (*$P<0.05$, **$P<0.01$)

dorsum run faster and had greater AMC. Also, larger males had higher body temperatures, and both male size and body temperature were positively related to blue chroma (Fig. 1). T-level direct (path coefficient=0.07) and indirect effects (through its effect on AMC, indirect path coefficient=0.09) on blue coloration were low (Fig. 1). Hence, we did not find evidence that T-level influences the blue coloration of males.

Collar

The lightness and size of the black stripes of male collar were related to male performance and immune response. Males with darker (i.e. lower lightness) and larger black collars had higher running speed and bigger AMCs (Fig. 2). Also, males with darker collar had greater endurance, produced a higher PHA response and had lower parasite loads (Fig. 2b). PHA response had a smaller positive effect on the size of the collar, and there was only an indirect effect of parasite load (indirect path coefficient through PHA response=0.11; Fig. 2a). Interestingly, larger males had larger black collars, but SVL had only an indirect effect on the lightness of the collar (indirect path coefficient through PHA response=0.34; Fig. 2b). Body temperature was positively related to T-level and had a positive effect on collar lightness and a negative effect on collar size: males with darker and larger black collars had lower body temperature (Fig. 2). We did not find evidence that T-level influenced the size or colour of the black collar.

The white area of the collar was only directly (direct path coefficient=0.36) related to AMC and indirectly to SVL (through its affect on AMC, indirect path coefficient=0.15). The white stripe was not related to other performance measurements, immune response, T-level or body temperature.

White spots

Males with more white spots were larger (number of spots, path coefficient=0.54; area of spots, path coefficient=0.40) and had bigger AMC (area of spots, path coefficient=0.44); yet, males with more white spots had lower endurance (path coefficient=−0.41) and lower body temperatures (path coefficient=−0.28). The number and total area of white spots on the male dorsum were not related to PHA response, parasite load, T-level or running speed.

Overall, path analyses suggest that different colour patches in Dickerson's collared lizard may convey moderately different information (Fig. 3). The black collar was related with indexes of performance, and these relationships were stronger than the relationships of performance with the blue dorsal colour. Moreover, lightness of the collar was related to immune status. White spots were only related to AMC (area) and endurance (number). Body temperature influenced the colour, especially the area of the black collar, and also affected PHA response. We found no evidence that T-level may be involved in colour display.

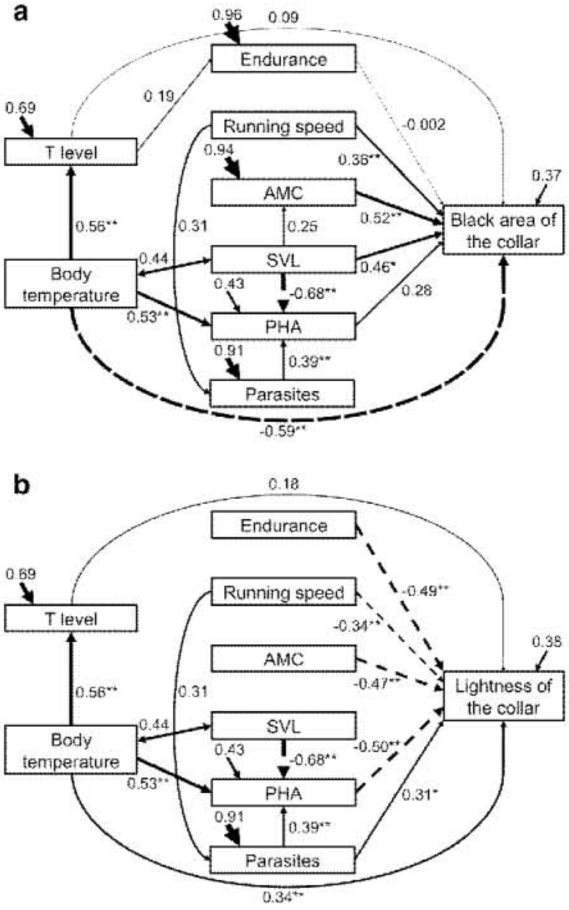

Fig. 2 Black collar indicates performance and immune response. Path model illustrating effects of performance measurements (endurance, running speed and AMC), immune response measurements (PHA-response and parasite load), SVL, T-level and body temperature on **a** size of the black collar (χ^2=15.43, *df*=18, *P*=0.63, AIC=852.26) and **b** collar lightness (χ^2=19.60, *df*=20, *P*=0.48, AIC=676.04). Thickness of the *arrows* indicates strength of the relation. *Double-headed arrows* indicate a covariance. *Single-headed arrows* indicate an effect of the variable at the base of the *arrow* over the variable at the head of the *arrow*. *Single-headed arrows* without a *box* at the base of the *arrow* indicate effects of unmeasured factors. *Solid arrows* indicate positive relations, whereas *dashed arrows* indicate negative relationships. Standardised estimate coefficients are given (*$P<0.05$, **$P<0.01$)

Finally, we evaluated whether colour traits were correlated with each other. After controlling for size, colour traits were not correlated with each other (all $P>0.05$), except for the correlations between the area of the black and white stripes of the collar ($R=0.44$, $P=0.003$), the number and area of the white spots ($R=0.47$, $P=0.001$), and a marginal correlation between the lightness of the collar and the number of white spots ($R=0.30$, $P=0.049$).

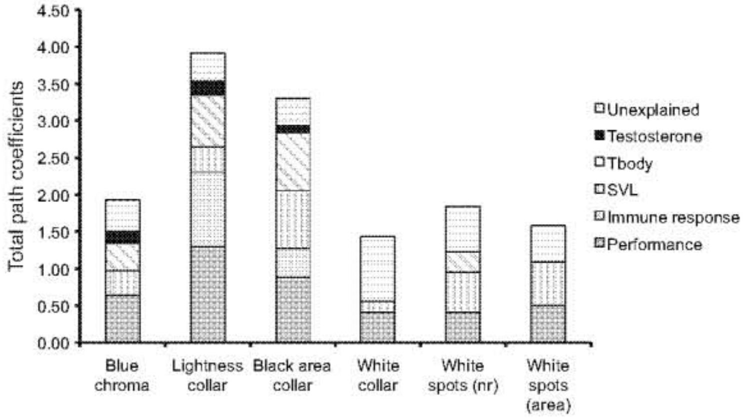

Fig. 3 Summary of path coefficients of the variables included in the models for the different colour traits. The total path coefficients for each variable were calculated as the sum of the absolute values of direct and indirect path coefficients. For visual simplicity, in the figure performance includes the added path coefficients of AMC, running speed and endurance, while immune response includes the added path coefficients of PHA response and parasite load

Parasite load and immune response

All ectoparasites found on males were mites of the family *Trombiculidae*. On average, males carried 175 mites, but there was a large variation among individuals (range 3–786). Although all ectoparasites were removed on the day of capture, roughly 7 days before the PHA test, animals with higher ectoparasite load had higher PHA response (Fig. 4a). Interestingly, bigger animals had lower PHA response (Fig. 4a, b). Effects of T-level on PHA response and parasite load were small (Fig. 4a). Furthermore, the model performed significantly better when testosterone was excluded (model including T-level AIC=567.37, model excluding T-level AIC=549.04; Wald test, $\chi^2=24.07$, $P<0.01$), suggesting that T-level had no significant effect on parasite load and PHA response.

Discussion

We found that in the Dickerson's collared lizard, multiple male colour traits indicate performance and immune condition. Male size, an important trait in determining dominance among lizard species (Tokarz 1985; Carpenter 1995; Sacchi et al. 2009), was positively correlated to the blue, the size of the black collar stripes and the number of white spots; the darkness of the collar was independent from size. However, independent of size effects, the results suggest that colour traits indicate male competitive ability and resource-holding power, while the blackness of the collar also indicates immunocompetence.

Dickerson's collared lizard males with deeper cobalt blue dorsums, larger and darker black collars and a greater number of white spots had larger AMC and performed better at the running speed test, and both of these performance traits are probably related to male competitive ability (Robson and Miles 2000; Perry et al. 2004; Huyghe et al. 2005; Husak and Fox 2006; Husak et al. 2006a, b). Among territorial male lizards, agonistic interactions with potential rivals include rapid pursuits to chase away males from territories and, when escalation occurs, fights with fierce biting that can produce serious wounds (Lappin and Husak 2005). The size of the AMC, the jaw-closing musculature, is directly related to maximum bite force (Lappin et al. 2006), and bite force predicts dominance and reproductive success (e.g. Herrel et al. 1999; Huyghe et al. 2005; Lappin and Husak 2005; Husak et al. 2006a). Hence, if running speed and bite force are key weapons in male-male competition, indicating their performance through colour displays may be advantageous to avoid the costs associated to fighting. In a closely related species, *C. collaris*, mouth-gaping displays by adult males provide an index of bite force during close-range encounters (<1 m; Lappin et al. 2006). Dickerson's collared males typically guard their territory from a prominent rock from where complex displays that may advertise their colour traits are exchanged with potential rivals. Our data suggests that multiple conspicuous body colour traits might relay information on male fighting ability from longer distances.

In our study, males with darker collars mounted a greater PHA response and had smaller parasite load, while males with larger black collars mounted a greater PHA response; no relationship between the blue of the dorsum and the number of white spots with PHA response or parasite load was found. Black colours normally depend on melanin (Jawor and Breitwisch 2003; Kuriyama et al. 2006), and melanin-based colour patches are often implicated in social communication and linked to the immune function (Macintosh 2001; Ducrest et al. 2008). The ability to mount a cell-mediated immune response, a generalised short-term response that promotes healing of wounds and enhances resistance to infections, may have important fitness consequences during the breeding season when male-male interactions increase the probability of injury (Zuk and Johnsen 1998). In the Dickerson's collared lizard, colour and size of black stripes of the collar of males

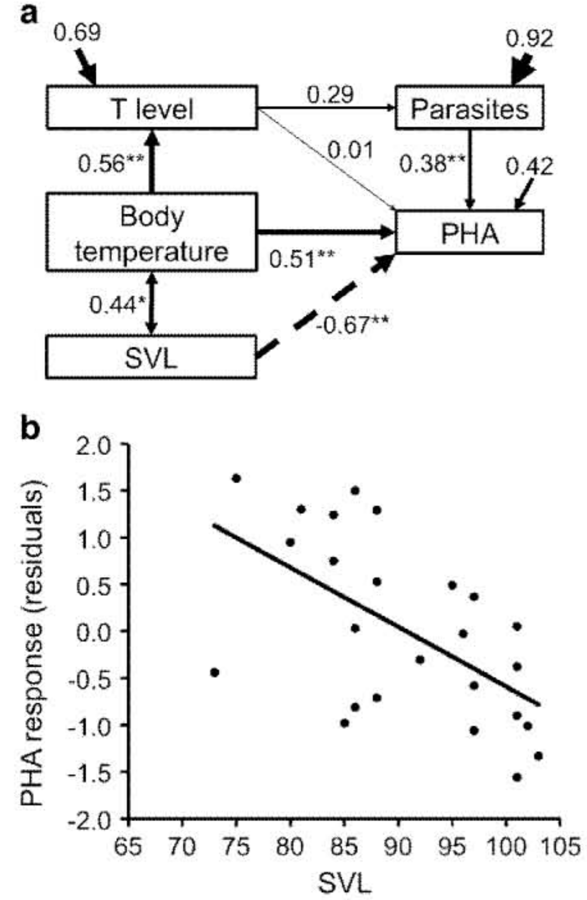

Fig. 4 **a** Path model illustrating effects of T-level and body temperature on parasite load and PHA response ($\chi^2=1.26$, $df=3$, $P=0.74$, AIC=567.38). Thickness of the *arrows* indicates strength of the relation. *Double-headed arrows* indicate a covariance. *Single-headed arrows* indicate an effect of the variable at the base of the *arrow* over the variable at the head of the *arrow*. *Single-headed arrows* without a *box* at the base of the *arrow* indicate effects of unmeasured factors. *Solid arrows* indicate positive relations, whereas *dashed arrows* indicate negative relationships. Standardised estimate coefficients are given ($^*P<0.05$, $^{**}P<0.01$). **b** The relationship between PHA response and size of the animal. On the y-axis, the standardised residuals of PHA response controlling for parasite load and body temperature are shown

are related to the strength of a cell-mediated immune response and parasite load, suggesting that the collar is a signal of the immune status of the male.

Interestingly, PHA response was stronger in male lizards that carried greater parasite loads. Similarly, a positive relationship between parasite load and an inflammatory immune response has been reported in two other lizard species: *Sceloporus jarrovii* (Goldberg and Holshuh 1992) and, only late in the reproductive season, in *Podarcis melisellensis* (Huyghe et al. 2010). A positive correlation between parasite load and immune response might result from a general priming effect, i.e. when previous immune challenges lead to an immune response that increases sensitivity of the immune system to subsequent challenges (Huyghe et al. 2010; Schmid-Hempel 2011). Hence, it is possible that due to a general priming effect, males with greater parasite load, and presumably whose immune system was recently challenged, elicited a stronger immune response, while individuals with lower parasite load elicited a weaker swelling response because their immune systems have not been equally primed (Huyghe et al. 2010). Interestingly, PHA response was strongly and negatively related to male size suggesting reduced immunocompetence with age (lizards grow during their entire life, hence size is likely related to age; Sexton et al. 1992). Future studies should investigate whether priming is of a general or specific type and whether immunosenescence occurs in Dickerson's collared lizards.

We found no evidence that plasma levels of testosterone influenced the expression of colour traits, enhanced performance or was correlated with PHA response or parasite load of Dickerson's collared lizards. Furthermore, our data did not support the immunocompetence handicap hypothesis as stated by Folstad and Karter (1992). In our study, all males had fully developed adult coloration and T-level determination was based on a single sample taken at capture. Mean level of plasma testosterone of males was within the range of values reported for other adult male lizards of similar size (e.g. Cox and John-Alder 2005; Baird and Hews 2007; John-Alder et al. 2009). Hence, it is unlikely that our estimations of T-levels were biased. In some lizard species, testosterone only influence colour during a critical period prior to or during colour development (Kimball and Erpino 1971; Hews et al. 1994; Hews and Moore 1996). It is possible that in the Dickerson's collared lizard, current male colour traits can therefore be unrelated to measured T-levels, but could instead reflect testosterone level during development or during a longer period of time. In addition, natural T-levels might fluctuate with stress, immune status at capture, or age (Folstad and Karter 1992; Moore et al. 1991). In our study, body temperature and T-level were strongly related, and body temperature and immune response were linked to size suggesting that individual variation in age and condition might be important to understand the potential trade-offs between the display of testosterone-dependent colour traits and physiological performance (Alonso-Alvarez et al. 2009; Ruiz et al. 2010). At present, we have no evidence that testosterone plays a role modulating colour traits or that compromises the strength of the inflammatory immune response.

Male body temperature influenced physiological variables and colour traits. Males with higher body temperature had a stronger PHA response and higher T-levels, and displayed bluer dorsum and smaller and lighter black collars. Body temperature was positively related to male size. In lizards, larger animals cool down at lower rates than smaller animals (Claussen and Art 1981). In our study, males were allowed to sunbath before temperature measurements, so it is unlikely that size-related differences in cooling rate have biased the estimation of body temperature. However, larger males probably have an advantage at maintaining their optimal body temperature than smaller males. In ectotherms, body temperature is a key trait for fitness due to its effects on growth, metabolism, immune response and locomotor performance (reviewed in Angilletta et al. 2002; Zimmerman et al. 2010). Furthermore, in lizards, temperature influences sexual colour traits (Bajer et al. 2012; Langkilde and Boronow 2012). Optimal body temperature in small ectotherms is mainly achieved by behavioural thermoregulation, which may be costly in terms of time invested and increased predation risk (Huey 1982; Dunham et al. 1989; Adolph and Porter 1993). Particularly, in species with very conspicuous colours that contrast with the natural background, as is the case of the Dickerson's collared lizard, visibility to predators during sunbathing will be high (Macedonia et al. 2009). In the Dickerson's collared lizard, predation pressure appears to be low and may not have a strong selection effect (Plasman et al. 2007). However, the extreme hot environment of the Sonoran Desert may impose important constraints and challenges for thermoregulation. Hence, individuals that are able to maintain body temperature within a range that optimises physiological performance, the display of colour traits and active time should enjoy greater fitness than those that are not. Therefore, in the Dickerson's collared lizard, colours dependent on temperature may signal current physiological and physical performance.

The exact mechanisms of how temperature affects integumentary colour in lizards are unknown, but changes in structural colours, such as blue, can result from altered spacing of the iridophores platelets in the cells of the light-reflecting layer (Morrison et al. 1996). Additionally, if melanin synthesis is temperature dependent, this may influence the intensity of melanin-dependent colours, such as the black of the collar, and structural colours due to its role as a purifier layer in structural colours (Quinn and Hews 2003; Grether et al. 2004). Future studies need to evaluate in more detail the influence of temperature on individual colour variation.

In conclusion, multiple colour traits of Dickerson's collared males indicate resource-holding power and immune condition; particularly, the black stripes of the collar might function as a status badge. Interestingly, most colour traits considered in this study were not related with each other, probably because of different physiological process involved in colour production. However, multiple traits that convey the same information may be selected for the advantages of reducing the time of assessment, the likelihood of erroneous assessments and cheating (Møller and Pomiankowski 1993; Candolin 2003). Future studies need to evaluate whether male multiple colour traits are used to assess the fighting prowess of potential rivals, to assess potential mates during mate choice or both. Furthermore, colours dependent on body temperature may advertise physical and physiological performance in ectotherms and therefore indicate status and condition. Coloration in male Dickerson's collared lizard can therefore be considered an honest signal that can be used to assess male performance and condition.

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Ethical standards The study complies with the current regulations of Mexico regarding animal welfare.

Conflict of interest The authors declare that they have no conflict of interest.

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Capítulo 3.

Effect of male colouration on female preference in the Dickerson's collared lizard

Chapter 3: Effect of male colouration on female preference in the Dickerson’s collared lizard

Melissa Plasman and Roxana Torres

Abstract

Females may increase fitness by choosing a mate of high quality (e.g. healthy, with good genes, and/or high competitive ability). Many species have evolved conspicuous traits that signal male quality and hence facilitating the selection process. Bright coloration is a common sexual trait in lizards and may signal quality in colourful individuals. In the Dickerson's collared lizard males have a blue body colour, a black and white collar and white spots on the dorsum. A recent study showed that bluer males and males with a bigger, darker collar have higher resource holding power (RHP) and males with bigger and darker black collar had a better immune response. Bluer males, but with smaller and lighter black collars had higher body temperatures. In this study we evaluated whether females prefer these conspicuous male colour traits. First, when females were presented with two males simultaneously we found that females spent more time near males with lighter black collar, but displayed more towards males with darker black collars. In a second test, females were simultaneously presented to three males who had their collar experimentally decreased, increased, or not manipulated. Females spent more time near males and displayed more to males with higher display frequency and un-manipulated or smaller collar size, but discriminated against males with enlarged collar. Independently of the treatment, females approached bluer males more. Overall, the results suggest that females based their mate preference on multiple signals. Females displayed more to males with darker black collars and approached bluer males more, thus they may prefer males that signal higher RHP. However, females approached and stayed more time near males with lighter and smaller collar, which may suggest that females prefer males which signal high body temperature. Body temperature is important for the physical and physiological performance of ectotherm animals and higher body temperatures in these males may indicate increased performance.

Keywords: mate choice, multiple signals, performance, immune response, temperature

Introduction

Mate choice is an important mechanism of sexual selection, affecting fitness of both males and females (Darwin 1871). Mate choice, typically female mate choice, is favoured when an individual benefits from mating with a high quality mate. These high quality mates might offer more direct benefits such as resources, a good territory, or lower probability of contagious diseases, and/or more indirect benefits, such as good genes that will be passed on to their offspring (Zahavi 1975, Hamilton and Zuk 1982, Andersson 1994). However, quality can rarely be observed directly, therefore sexual selection has favoured the evolution of traits that indicate quality. Hence, high quality males that display more elaborate sexual signals obtain higher fitness, as female prefer them as mates or they can manipulate females into choosing them (Andersson 1994, Kokko et al. 2003, Parker 2006).

In lizards, female mate choice was thought to be absent (Noble and Bradley 1933), and more recent reviews suggest that it still appears to be rare (Olsson and Madsen 1995, Olsson et al. 2013). Typically, female lizards choose a territory and mate with the male owner (Kiester 1979, Stamps 1987, Tokarz 1998), and return to the same territory as previous years (Tokarz 1995), even if the resident male changes or is experimentally replaced by a subordinate male (Stamps 1983). Additionally, Stamps (1983) stated that female lizards would rarely be courted by more than one male, as males are highly territorial. However, females may temporarily leave their home range to mate with a high quality male, when their home range overlaps with only low quality males' territories (Calsbeek and Sinervo 2002, Vitousek et al. 2007). Also, in many lizard species males have patches of bright colours (reviewed in Cooper and Greenberg 1992), which are emphasized by behavioural displays during courtship (Cooper and Greenberg 1992, Martins et al. 2014) and may be used to attract and stimulate receptivity in the female (Halliday 1992). Hence, although mate choice is expected to be rare among lizard species (Olsson and Madsen 1995, Olsson et al. 2013), female lizards have been found to mate selectively with bigger males (Cooper and Vitt 1993, Tokarz 1995), distinguish between male pheromones (Martín and López 2000; Huyghe et al. 2012) and prefer brightly coloured males over duller males (Baird et al. 1997, Kwiatkowski and Sullivan 2002, Bajer et al. 2010; but see Olsson and Madsen 1995).

Colour patches can signal the quality of the individual and thus communicate the benefits that may be obtained by choosing this particular male as mate. For example, colourful displays make the male more conspicuous to predators and other males, probably reducing survival (Moore and Marler 1987, Martín and Forsman 1999, Martín and López 2001). Thus bright colours could be considered a handicap and only highly skilled males will be able to survive with this trait (Zahavi 1975, 1977). Additionally, colour patches can indicate the male's condition, performance, health, or good genes (Hamilton and Zuk 1982, Folstad and Karter 1992; for examples in lizards see: eg. Stapley and Whiting 2006, Martín and López 2009, Fitze et al. 2009, Huyghe et al. 2009, Langkilde and Boronow 2010, Bajer et al. 2011, Molnár et al. 2012). On the other hand, black colour patches are often used as status badges (Jawor and Breitwisch 2003). Females may choose dominant males, because her sons may inherit the competitive abilities (Wong and Candolin 2005). Female *Sceloporus undulatus,* for example, prefer males with a black colour patch in the throat, and additionally she evaluates other morphological aspects that affect dominance (Swierk et al. 2012).

According to the multiple messages hypothesis (Møller and Pomiankowski 1993) different ornaments can signal different characteristics that affect fitness. These ornaments can include different colour patches or even different aspects of the same patch (like chroma, lightness, size of the patch or number of patches; Candolin 2003, Grether et al. 2004). Hence, a female could base her preference on colour pattern rather than just one colour patch. For example, in the lizard *Uta stansburiana* there are three morphs (in males and in females) for throat colour, and also three morphs in the colour pattern of the dorsum (striped, barred or chevrons). All combinations of throat and dorsum morphs exist, but whereas throat colour indicates reproductive strategy, survival depends on the combination of throat colour and dorsal pattern, and females base their preference on the combination (Lancaster et al. 2009). On the other hand, multiple signals may also convey the same information according to the redundancy hypothesis (Møller and Pomiankowski 1993). This may reduce errors in the assessment and make cheating more difficult (Møller and Pomiankowski 1993, Candolin 2003). Furthermore, the frequency or intensity of behavioural displays may indicate performance measurements of the males (Brandt 2003, Husak 2004). Hence, display, including both behaviour and colour, may be a complex multiple signal towards the female that might include a single or multiple messages.

Dickerson’s collared lizard (*Crotaphytus dickersonae*) males have a striking cobalt blue colour over their entire body. Females are brown, with yellow legs and tail. Females form orange spots on their sides when they are receptive (Cooper and Furgunsen 1971), which elicit courtship behaviour in males (Baird 2004). Both sexes have a collar of two black stripes, divided by a white stripe, and white spots on the dorsum. The striking colouration makes the males of Dickerson's collared lizard very conspicuous, and males are easily seen in the open habitat of the Sonoran desert. Males are 14% larger and 63% heavier than females (Plasman et al. 2007). Males are very territorial and home range overlap is probably small (Baird 2013b). Still, as they often display at high perches males are visible over long distances and females may evaluate several males simultaneously and choose between them. These displays may emphasize their colouration (Baird 2013a), and males display to both males and females (pers. obs.). In an earlier study it was found that in the males, a more intense blue dorsal colour and a bigger and darker black collar relate with higher resource-holding power (RHP), and a bigger and darker black collar also indicates better immune response (Plasman et al. 2015). Hence, male coloration may signal dominance and good genes. In this study we evaluated whether females use male colouration when selecting a mate. We expected females to choose bluer males with bigger and darker collar, as females may then give rise to dominant sons. Also, by choosing males with a bigger darker black collar offspring may inherit disease resistant genes. Simultaneously, females may obtain more direct benefits such as a lower chance of parasite infections and the benefits of a high quality territory. The ample benefits that may be obtained by choosing a male with darker and bigger black colour suggest that for the females this might be an important trait to evaluate. Therefore, in a second test we manipulated collar size and evaluated female's preference, expecting her to prefer the male with an increased collar area.

Methods

To determine whether Dickerson's collared lizard females prefer specific colour traits in males, preference experiments were performed during the reproductive periods (May to July; McGuire 1996) of 2011 (experiment 1) and 2012 (experiment 2). Experiments took place in Bahía de Kino in the state of Sonora in Mexico, within the distribution range of Dickerson's collared lizard for which temperature and light-day rhythms were normal for this species. A total of 43 adult males and 36 adult females were captured and were kept in individual outdoor cages. Lizards were given water *ad libitum*, and each morning cages were misted and lizards were given a live cricket.

On the day of capture, from each lizard snout vent length was measured with a ruler (± 1 mm) and mass (± 0.1 g) was measured with a PESOLA scale. Further, after males had been allowed to sunbath for at least an hour in the afternoon (between 16:00 and 18:00 hours), colour measurements were taken with a spectrophotometer (MINOLTA CM-2600d) that measures reflectance at 10 nm intervals from 360 to 740 nm. Even though these lizards can perceive UV, the blue dorsal colour and the black collar have low reflectance in the UV range (Macedonia et al. 2009). Measurements of the blue colour were taken on three places on the dorsum. Reflectance curves of the three measurements were averaged and blue chroma was calculated as reflectance from 400-480 nm/total reflectance. One measurement was taken of each of the black stripes of the collar, reflectance curves were averaged and lightness was calculated as the total reflectance. Photos of males were taken to estimate the area of the collar and the area and number of white spots (Adobe Photoshop version 8.0). Lizards were placed flat on a white flat surface and photographs were taken from both lateral sides and dorsum. Photographs included a ruler for scale. Females used in these test all displayed orange spots, which indicate fecundity (Cooper and Furgunsen 1971). Each female was only used once in a preference trial, whereas males were used in 1 to 3 trials in the first experiment and from 1 to 6 trials in the second experiment. Lizards entered to the trials an average of 4.5 ± 1.9 days after capture in 2011 and 4.9 ± 3.6 days after capture in 2012. Experiments were performed during the hours of highest activity 10:00 to 14:00 and 16:00 to 18:00 hours.

Experiment 1

In a first experiment we evaluated whether females displayed a preference for certain aspects of the natural male colouration. Experiments took place in a setting in which the female could observe (but not smell), approach and display to two males simultaneously. These enclosures were kept at 37ºC, which is the average body temperature as measured in the field (Plasman et al. 2007). The enclosures were made from glass, to avoid flow of pheromones and for easy cleaning between trials. Males were kept in two adjacent enclosures (40 x 40 x 40 cm). White paper between these enclosures avoided visual contact between the males. The female was put in an enclosure in front of the two enclosures of the males (80 x 40 x 40 cm) where she could observe them simultaneously. In the female enclosure a line was drawn at 10 cm from the glass that separated the female from the males, to differentiate a preference area in which the female is near the male and which was visible in the video recordings. In each trial males were of similar snout vent length (difference of less than 2 mm), but differed in blue chroma (average blue chroma = 0.287 ± 0.025 SD; mean difference between males in a trial = 0.026 ± 0.023), size of the black collar (average size = 2.95 ± 0.75 cm^2; mean difference between males in a trial = 0.54 ± 0.35 cm^2), lightness of the black collar (average lightness = 74.59 ± 32.04; mean difference between males in a trial = 24.59 ± 16.04), and number of white spots (average number = 159 ± 34; mean difference between males in a trial = 42 ± 27), which had been measured prior to the experiment. Males and females in each trial were from different populations to avoid possible effects of familiarity. Males were randomly assigned to either the left or right male enclosure. Posterior, the female was placed in the female enclosure and lizards were allowed to adapt for 20 minutes while a brown canvas screen between the males and the female avoided visual contact. After the screen was removed, trials lasted 30 min. Trials were recorded with a video camera (Sony Digital 8 Handicam), and the behaviour of both males and females was later registered from the videos. From female behaviour we registered (1) preference time as the time (in sec) the female spent in the preference area, (2) approach as the time (in sec) she actively approached the male, scratching the glass, and (3) the absolute occurrence of all displays performed by the female to the male which included push ups, full show (standing high on the legs while having the throat inflated), walking high on the legs, and putting her head close to the head of the male nearly touching the glass. Although full show performed by the female has been considered rejection behaviour in some lizard species (Bleay and Sinervo 2007), in collared lizards it is part of the display repertoire of courtship behaviour (Baird et al. 2003, Baird 2004). Male behaviour was recorded as (1) approach, the time the male tried to actively approach the female, scratching and jumping up to the glass, (2) number of displays which included head bobbing, dewlapping, extending of the dewlap, push ups (male flexes front legs), raises (male extends the front legs), full shows (arcing the back and extending the gular area), circling on the spot, opening of the mouth, and walking backwards. Separate displays were correlated ($R > 0.27$, $P < 0.04$); hence all displays were added and included as total number of displays in the models. Trials were performed with 16 different females and 23 males. In four trials females did not display a preference for either of the males. Compared to females that choose, females that did not choose tended to be smaller (SVL = 78 ± 4 and 68 ± 8 mm resp., $t = -2.17$, $P = 0.11$, $df = 3.44$) and weighed less (19.8 ± 4.7 and 12.1 ± 4.9 g resp., t = -2.57, P = 0.05, df = 4.99). These trials were excluded from the analyses, so the final sample size of experiment 1 was 12 trials.

Experiment 2

To evaluate female preference for the black collar of males we performed a second experiment in 2012, in which collar size was manipulated. This experiment took place in the similar settings as experiment 1, however instead of two males the female could observe, approach and display to three males simultaneously during 60 min. The female enclosure was of the same size as in experiment 1. Male enclosures, as there were now three adjacent enclosures, were slightly smaller (26 x 40 x 40 cm). In a trial the females could observe one male with increased collar, one male with decreased collar and one male in which collar area was not manipulated. Males were randomly placed in one of the three male enclosures. For each trial three males of similar snout vent length (difference of less than 2 mm; ANOVA: $F_{1,55}$ = 0.02, P = 0.88) were randomly assigned to a treatment group in which the size of the collar was manipulated with a non-toxic acrylic paint. In the group in which the collar was increased, all three bands (two black and one white) were increased by approximately 40% and in the reduced collar size group area were reduced by approximately 25%. In the control group collar was painted to control for change in lightness of the collar, but size was not manipulated. Final black collar area was on average 6.25 ± 1.48 cm^2 for the increased treatment group, 4.01 ± 1.12 cm^2 in the control group and 2.84 ± 0.83 cm^2 in the reduced treatment group. Males in the reduced collar area maintained collar size within natural range of black area (natural range = 1.22 - 4.89 cm^2), but males in the increased collar treatment had collars that were slightly bigger than natural. Paint affected lightness of the collar, but did not differ between treatments (ANOVA: $F_{1,55}$ = 0.025, P = 0.88; lightness [% of reflectance] of increased collar = 85 ± 45, reduced collar = 83 ± 42 and control = 73 ± 41). As colour may be more dynamic as we formally thought, for more accurate determination of colour as perceived by the female, male colour measurements of blue chroma and lightness of the black collar (as described earlier) were taken immediately before and after each trial. Trials were performed with 20 females and a total of 20 males. In one trial the female did not display a preference for any male, this trial was removed from the analyses. Trials were recorded with a video camera and behaviour was registered in the same manner as for experiment 1.

Statistics

Data were analyzed using General Linear Mixed Models (GLMM) in R 3.2.0 (package pscl). From the first experiment separate models were performed to analyse (1) preference time, (2) approach, and (3) the total number of displays of the female towards the male. Because females often did not respond to a certain male, our data included many zeros, therefore data was analysed with zero-inflated models with negative binomial distribution, which functioned better than zero-inflated models with poisson distribution or generalized linear models with poisson distribution (vuong test: $P < 0.01$). Zero-inflated models included female identity as random factor, and male colour variables (blue chroma, lightness of the black collar, area of the collar, number and area of the white spots) and male behaviour as fixed effects. Additionally, male identity was added as a random factor to adjust for possible effects of using some males multiple times. Male behaviours included were either approach or total number of displays.

For the second experiment similar GLMMs were performed, but treatment was added as another fixed factor, and interactions between treatment x colour measurements (eg. blue chroma, black area of the collar, lightness of the collar, number of white spots) and treatment x number of male displays were tested. Although males were randomly assigned to the treatments, we found that males in the treatment of increased collar area were significantly heavier (44.37 ± 3.26 g), than control (38.22 ± 2.89; Wilcoxon: $V = 188$, $P < 0.001$), or males with decreased collar area (40.12 ± 5.73; Wilcoxon: $V = 144$, $P = 0.047$). Furthermore, males in the control group had more white spots (203 ± 19), than males in the increased treatment group (171 ± 18; Wilcoxon: $V = 3$, $P < 0.001$), and decreased treatment (181 ± 16; Wilcoxon: $V = 170.5$, $P = 0.001$). Therefore, these variables were always included in the models. The best models were selected based on Akaike Information Criterion (AIC). When several models had similar AICs (difference of less than 2), the most parsimonious model was chosen.

Results

Experiment 1: Do females prefer more colourful males?

Female behaviour: Females spent more time near the male and approached the male more when the black collar of the male was lighter, yet displayed more to males with a darker black collar (table 1; fig 1 and 2). Females also spent more time near a male with fewer white spots and approached more to males with a smaller white collar (table 1). We did not find any relationship between female's behaviour and the blue dorsal colour of the male. Females approached the male more often when the male displayed more (table 1). Additionally, heavier females displayed more (Spearman correlation test: $R = 0.59$, $P = 0.001$, $n = 32$), and females that spent more time near the male also displayed more ($R = 0.53$, $P = 0.002$, $n = 32$).

Male behaviour: In the trials, the behaviour of males was not related to their blue dorsal colour, or lightness and size of the black collar ($P > 0.08$). However, heavier males, and males with a bigger area of white spots displayed more ($R = 0.40$, $P = 0.03$, and $R = 0.58$, $P < 0.01$, $n = 32$ respectively for weight and white spot area).

Experiment 2: Do females prefer males with larger collars?

The size manipulation of the male’s collar affected the time females spent near a male (table 2). When the frequency of male displays was considered, females spent more time and displayed more to males with decreased or non-manipulated collars, whereas females spent less time near and displayed less to males with increased collar (fig 3 and 4). The collar size manipulation did not have a significant effect on the time the female approached the male (table 2), yet females approached males more when they had higher blue chroma (fig 5). Also, females approached males more when they had more white spots and were heavier (table 2).

Discussion

Males of Dickerson's collared lizard display a striking coloration. Our results suggest that female mate preference was based on a combination of phenotypic traits and behaviour that indicate the males’ competitive and physiological performance. In the first series of trials in which pairs of males, that naturally differed in some colour traits, were presented, females spent more time near males with lighter black collars but displayed more towards males with a darker collar. When collar size was manipulated, females spent more time near and displayed more to males with reduced or un-manipulated collar size, discriminating against those males with an enlarged collar. Although in the first experiment no relation was found with the conspicuous blue colour, in the second experiment females approached bluer males more.

A bigger and darker black collar and a more intense blue dorsal colour signal higher running speed and bite force, whereas the darkness of the collar is also related to lower number of parasites and better immune response and could thus be considered indicators of high quality males (Plasman et al. 2015). Females approached bluer males more and displayed more to males with a darker black collar. Females that prefer to mate with these males may obtain dominant offspring (Berglund et al. 1996), since both colour (reviewed in Hubbard et al. 2010 and for lizards in Olsson et al. 2013) and dominance (e.g. Moss et al. 1985, Moore 1990, Wilson et al. 2011) are likely inherited. In the Eastern collared lizard, *C. collaris*, when, in a population, colour brightness related with RHP, females also preferred brighter males (Baird et al. 1997). Moreover, by preferring males with darker black collars females might obtain direct benefits by avoiding males with high parasite loads and indirect benefits, if male’s immune capacity is inherited to the offspring

(Hamilton and Zuk 1982). Nevertheless, females stayed more time near males with a lighter collar, and contrary to our prediction, when collar size was manipulated, females discriminated against males with a collar that was increased in size. Because collars in the increased group were slightly larger (28%) than the natural variation it is possible that females did not recognise these males as conspecifics. However, this is unlikely because the collar is just a small part of the complex male colouration, furthermore there are no similar species within their distribution range. Hence, females may be spending more time near males with lighter and smaller collars and displayed more to males with smaller or unmanipulated collars to avoid males with high RHP. Females may prefer males with lower bite force (Huyghe et al. 2012), because males may bite the females during copulations (Baird et al. 2003), and they may try to avoid the costs of copulation (Qvarnström and Forsgren 1998, Lessell 2005).

Alternatively, females may be evaluating other qualities. For example, a lighter or smaller black collar may signal age, which may be important to evaluate as this affects male reproductive strategy (Baird et al. 2003a). Furthermore, reproductive immaturity and senescence may occur, and females may prefer middle-aged males (Richard et al 2005). In Dickerson's collared lizard, black collar lightness did, however, not correlate with body size, but black collar area did increase with body size (Plasman et al. 2015) and may indicate age in this species with undetermined growth (Sexton et al. 1992). Thus females may be avoiding old males.

Interestingly, a more intense blue colouration and a lighter and smaller black collar all appear to signal higher body temperatures (Plasman et al. 2015). High body temperatures in ectotherms can increase physical and physiological performance (Angilletta et al. 2002) and in Dickerson's collared lizard increased immune response (Plasman et al. 2015). Hence, these colour traits may signal male quality to the females. Normally darker colours are related with higher body temperatures in lizards (Clusella-Trullas et al. 2007), opposite of the findings in this species. Collar area is small and its heath absorption may be limited. The intensity of the blue colour on the other hand was also higher in warmer animals in other species (Morrison et al. 1996, Hettyey et al. 2009, Langkilde and Boronow 2012). Furthermore, high body temperature increased the intensity of the blue colouration and mating chance in male Moore frogs, *Rana arvalis* (Hettyey et al. 2009). The intensity of the blue colour may then fluctuate with body temperature, as it does in the Eastern fence lizard (Langkilde and Boronow 2012). This may be the reason for which we did not find female preference for the blue male colour in experiment 1, as in that experiment blue colour was measured several days before the trial, on the day of capture, instead of immediately before the trial as was done in experiment 2.

Female lizards may base their mate preference on multiple signals, evaluating several male traits simultaneously (Lancaster et al. 2009, Swierk et al. 2012). In our study we found that females evaluated blue dorsal colour and the black collar. These colour traits may signal similar qualities, like RHP and body temperature, yet they differ in that a more intense blue colour signal both higher RHP and higher body temperature, and a darker and bigger black collar signals higher RHP but lower body temperature. Our results indicate that females prefer males with higher body temperatures to males with higher RHP. Furthermore, male display frequency also affected female behaviour. Females preferred males with higher display frequency. In the Eastern collared lizard, males with higher display frequency had increased mating success (Baird et al. 2007). Behavioural displays may be dynamic signals of male performance. For example, head bobbing has been found to relate with endurance (Leal 1999, Perry et al. 2004). However displays may also accentuate colour traits (Candolin 2003, Baird et al. 2003) and facilitate distinction between males (Hebets and Papaj 2005). In our experiments male displays appear to emphasise collar area as females discriminated against males with increased collar size only when these males displayed frequently.

Although intra-sexual selection may be important for male mating success in lizards (Zamudio and Sinervo 2003, Husak et al. 2006, Baird et al. 2007), female preference for certain male traits have also been shown in lizards (Cooper and Vitt 1993, Tokarz 1995, Martín and López 2000; Huyghe et al. 2012), and include the selection of colour traits (Baird et al. 1997, Kwiatkowski and Sullivan 2002, Bajer et al. 2010). Yet female choice may be difficult to unravel as females probably evaluate several traits simultaneously (Lancaster et al. 2009, Swierk et al. 2012) and preference may also depend on her own status, age or genes (Moore and Moore 1991, Olsson et al. 2003, Mays and Hill 2004, Hunt et al. 2005, Richard et al. 2005, Suk and Choe 2008, Holveck and Riebel 2009, Lancaster et al. 2009, Laloi et al. 2011). Also, where it might be beneficial for a male to signal RHP in male competition, females may dislike these traits; thus sexual conflict takes place. Although it is not apparent which male qualities the females favour, in Dickerson's collared lizard females preferred males with lighter and smaller collars, but with a more intense blue colouration, suggesting that they prefer hotter males.

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Tables

Table 1. Female preference for male colour traits. Female preference was estimated as preference time, approach, and the number of female displays directed towards the male. The analyses were performed using zero inflated GLMM with negative binomial distribution. Initial models included blue chroma, lightness of the black collar, area of the black collar, area of the white collar, and number of displays of the male. Best models are shown.

Preference Time	estimate	95%CI (lower, upper)	z	P
Fixed effects				
Lightness of black collar	0.037	(0.012, 0.062)	2.877	0.004
Number of white spots	-0.022	(-0.043, -0.001)	-2.102	0.036
Variance components				
Female ID	-0.119	(-0.263, -0.025)		
Male ID	0.096	(-0.022, 0.214)		
Female Approach				
Fixed effects				
Lightness of black collar	0.008	(0.000, 0.015)	1.889	0.059
White collar area	-4.336	(-6.657, -2.016)	-3.663	<0.001
Displays	-0.013	(-0.025, -0.001)	-2.075	0.038
Variance components				
Female ID	-0.041	(-0.181, 0.099)		
Male ID	0.073	(-0.048, 0.194)		
Female Displays				
Fixed effects				
Lightness of black collar	-0.012	(-0.025, 0.000)	-1.897	0.058
Approach	0.008	(-0.002, 0.017)	1.594	0.111
Variance components				
Female ID	-0.050	(-0.187, 0.087)		
Male ID	0.049	(-0.065, 0.162)		

Table 2. Female preference for different male colour traits when the size of the male's collar is manipulated. Female preference was estimated as preference time, approach, and the number of displays performed by the female directed towards the male. The analyses were performed using zero inflated GLMM with negative binomial distribution. Initial models included treatment, mass, blue chroma, lightness of the black collar, area of the black collar, area of the white collar, and number of displays of the male. Best models are shown.

Preference Time	estimate	95% CI (lower, upper)	z	P
Fixed effects				
Treatment				
Intercept	4.311	(-6.114, 14.737)	0.810	0.418
Increased collar	1.688	(-0.224, 3.600)	1.731	0.083
Reduced collar	-1.351	(-3.379,0.676)	-1.307	0.191
Displays	0.021	(-0.020, 0.062)	1.001	0.317
Increased collar x Displays	-0.074	(-0.131, -0.016)	-2.516	0.012
Reduced collar x Displays	0.021	(-0.031,0.074)	0.803	0.422
Mass	0.036	(-0.157,0.084)	0.602	0.547
Number of white spots	-0.001	(-0.036.0.033)	-0.070	0.944
Variance components				
Female ID	-0.026	(-0.109,0.057)		
Male ID	-0.001	(-0.063,0.065)		

Female Approach	estimate	95% CI (lower, upper)	z	P
Fixed effects				
Treatment				
Intercept	-19.545	(-29.896, -9.194)	-3.701	<0.001
Increased collar	1.371	(0.181, 2.562)	2.258	0.024
Reduced collar	0.680	(-0.425, 1.785)	1.206	0.228
Blue chroma	28.658	(13.304, 44.013)	3.658	<0.001
Mass	0.107	(0.007, 0.209)	2.088	0.037
Number of white spots	0.050	(0.026, 0.074)	4.138	<0.001
Variance components				
Female ID	-0.003	(-0.065, 0.059)		
Male ID	-0.035	(-0.081, 0.011)		

Female Displays	estimate	95% CI (lower, upper)	z	P
Fixed effects				
Treatment				
Intercept	-3.710	(-10.265, 2.854)	-1.109	0.267
Increased collar	0.621	(-0.576, 1.818)	1.017	0.309
Reduced collar	-0.003	(-1.349, 1.343)	-0.004	0.996
Displays	0.038	(0.007, 0.069)	2.380	0.017
Increased collar x Displays	-0.044	(-0.076, -0.012)	-2.667	0.008
Reduced collar x Displays	-0.007	(-0.045, 0.031)	-0.461	0.718
Mass	0.096	(0.019, 0.173)	2.459	0.014
Number of white spots	0.005	(-0.016, 0.026)	0.466	0.641
Variance components				
Female ID	3.383	(-16.001, 22.567)		
Male ID	-13.713	(-102.668, 75.242)		

Figures

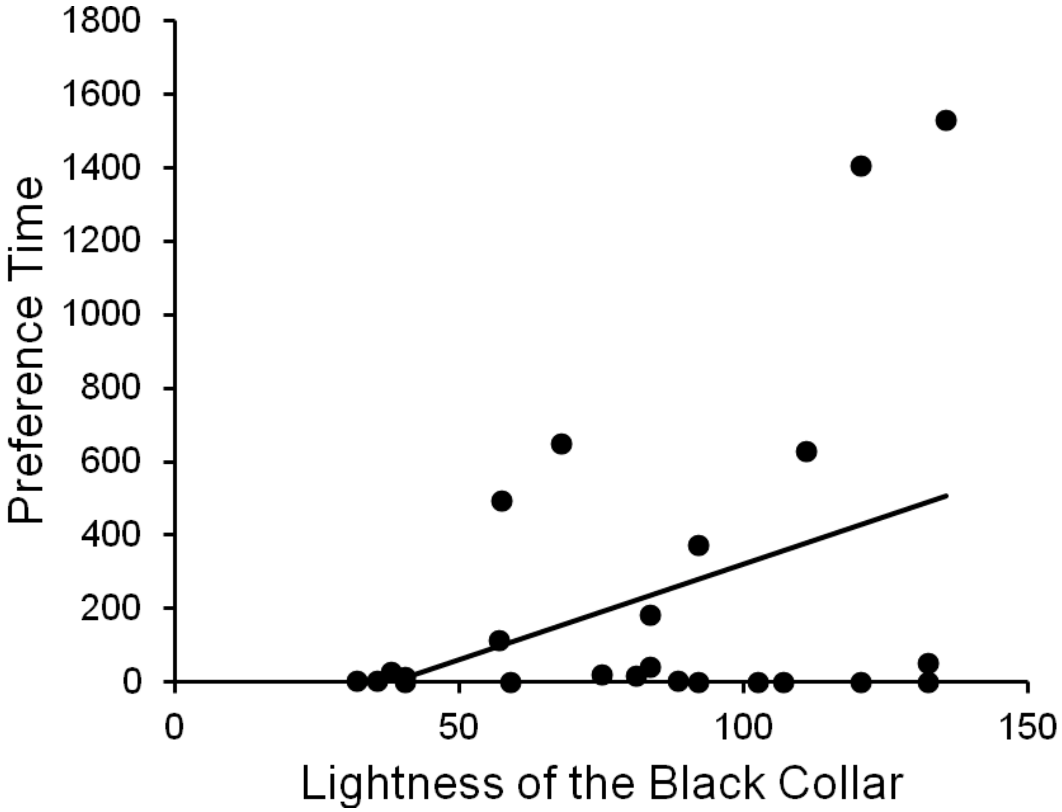

Figure 1. Preference time (in sec) of the female in relation with the lightness of the male’s black collar.

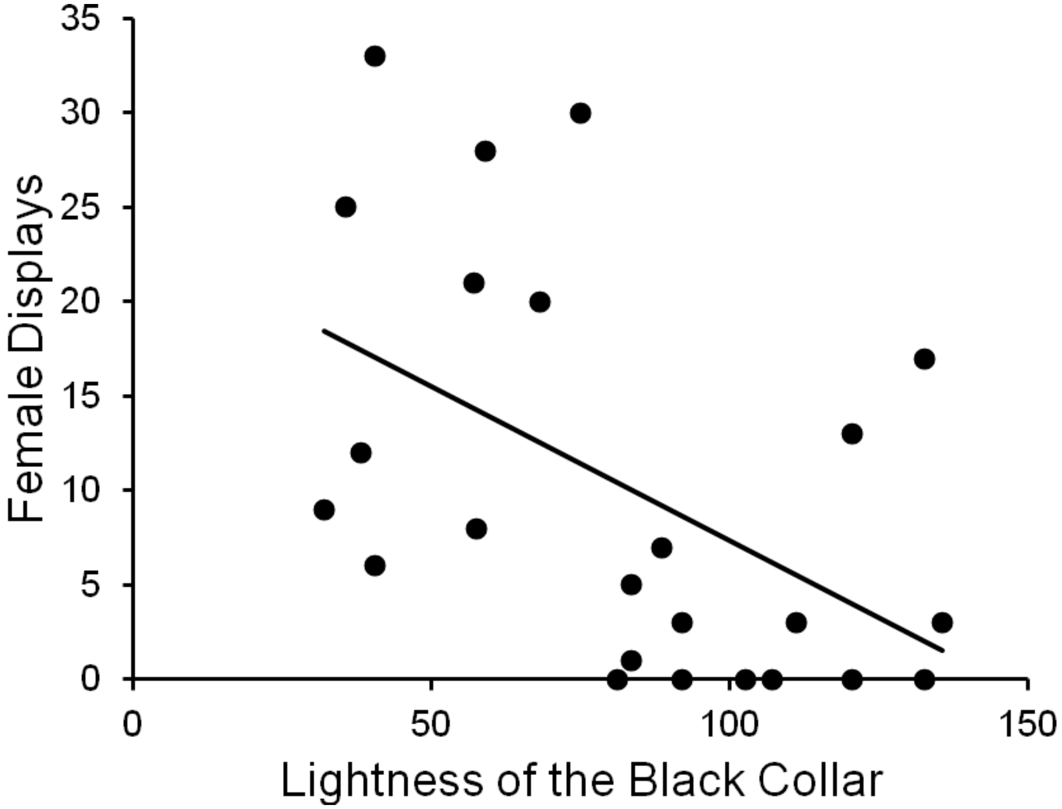

Figure 2. The total number of displays performed by the female towards the male in relation to the lightness of the black collar of the male.

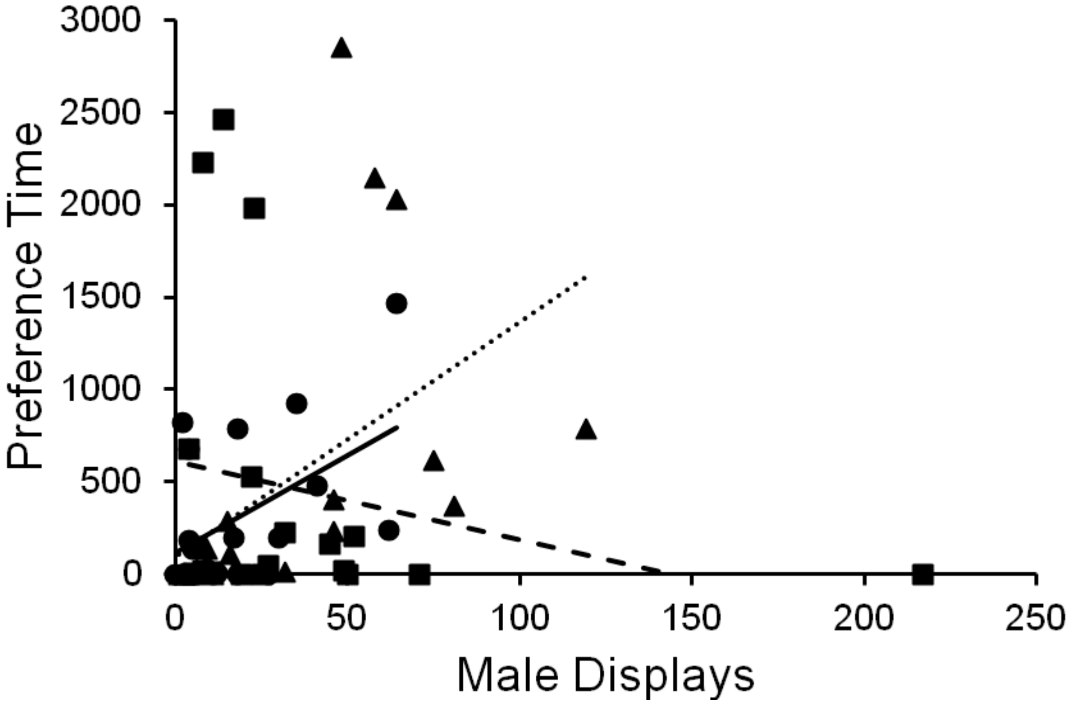

Figure 3. The relation between the preference time of the female and the total number of displays made by males with increased collar area (squares and dashed line), control males (round dots and solid line), and males with decreased collar area (triangles and dotted line).

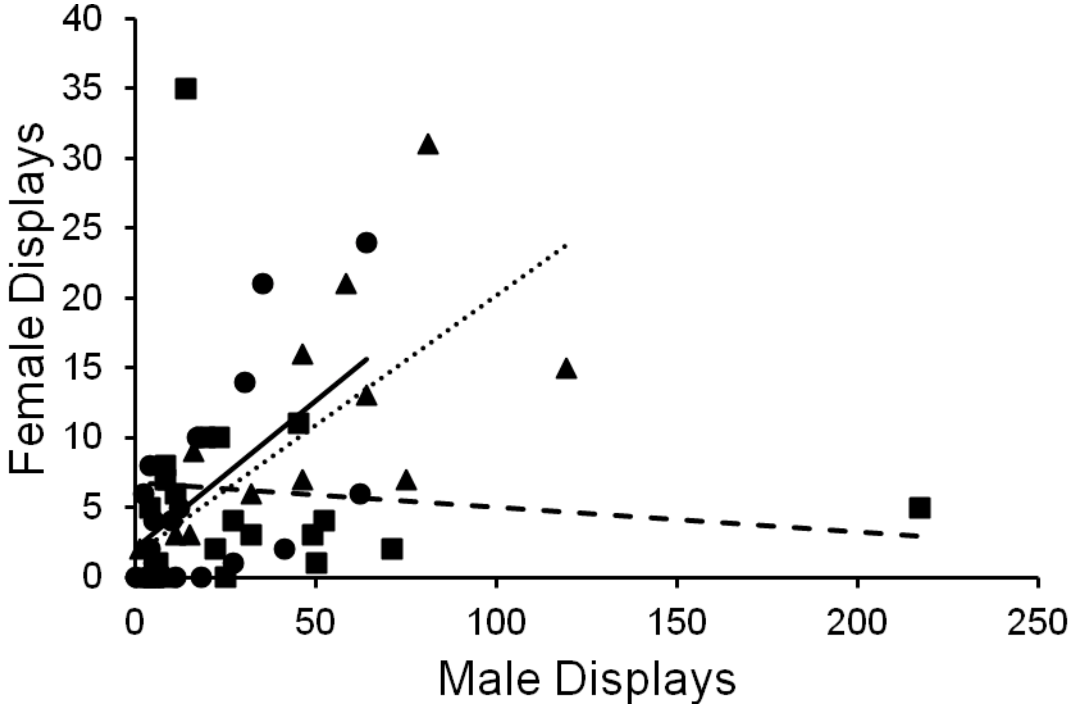

Figure 4. The relation between female displays and the total number of displays of males with increased collar area (squares and dashed line), control males (round dots and solid line), and males with decreased collar area (triangles and dotted line).

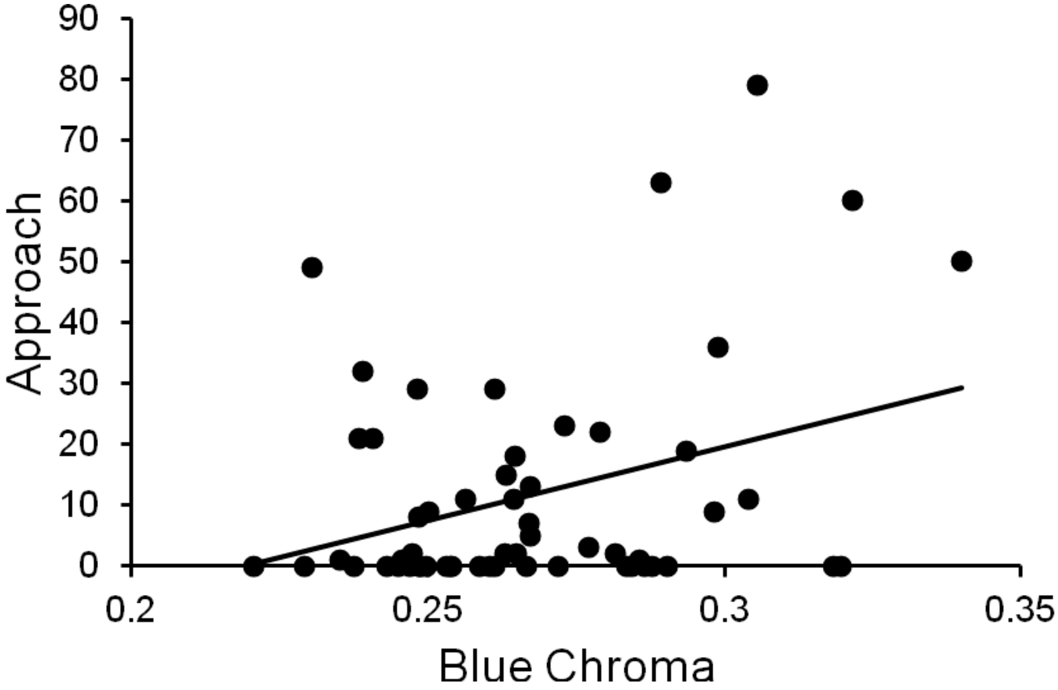

Figure 5. The relation between the approach of the female (in sec) and the blue chroma of the male (measured just before the trial).

Capítulo 4.

The use of male colouration as signal of resource holding power in Dickerson's collared lizards

Chapter 4: The use of male colouration as signal of resource holding power in Dickerson's collared lizards.

Melissa Plasman and Roxana Torres

Abstract

Many lizard species are highly territorial and costly fights are common to acquire territories. Signals that indicate fighting ability may reduce these costs, as animals can anticipate the outcome and only initiate a fight when the chance of winning is high. In many lizard species males have conspicuously coloured patches that are displayed during male-male encounters. Dickerson's collared lizard, *Crotaphytus dickersonae*, males are striking blue, with white spots on the dorsum, and they have a black and white collar. Bluer males and males with a bigger, darker collar have higher running speed and bite force, traits related to resource holding power. In this study we evaluated whether males use the colouration of their opponent as signal of its resource-holding power and adjust their behaviour accordingly. Staged interactions were filmed and behaviours recorded. In a second set of trials, male collar area was manipulated to be either big or small. We found that no single colour trait could predict the outcome of the fight. However, dominant males were more aggressive towards bluer males. Further, subordinate males displayed more submissive behaviour when their opponent had a smaller collar, even though a smaller collar signals lower resource-holding power. We conclude that the male's behaviour is affected by the opponent's coloration, yet they also evaluate other traits. Interestingly we found that in some trials the subordinate male strongly reduced its blue coloration and turned brown. We suggest that this "loser effect" may occur to reduce aggression by the dominant male.

Keywords: male-male competition, multiple signals, colour change, loser effect

Introduction

In many lizard species males fight to acquire territories (Stamps 1983, Pianka and Vitt 2003). Although territories might be necessary to obtain food and refuges, they are also frequently essential to gain mates, and the male's territory normally overlaps several female home ranges (Lappin and Husak 2005). Therefore fitness benefits of acquiring and defending a territory may be high. Even so, the costs of fighting to maintain a territory may be also high. During fights animals lose time and energy, they are conspicuous to predators and simultaneously less alert, and they may be injured (Marler and Moore 1988, Olsson 1994, Martín and López 2001). Hence, it will be beneficial for both opponents to settle the conflict avoiding high costs (Maynard Smith and Price 1973). Signals that indicate male fighting ability may lower the costs of fighting, as males can evaluate the possible outcome, and only initiate a fight when the chance to win is high (Maynard-Smith and Price 1973, Rohwer 1982, Olsson 1994). Fights will then only escalate when opponents have similar resource-holding power (RHP) or dominance status, and outcome may depend on differences which are not indicated by the signal, like fighting experience or motivation (Schwartz et al. 2007; Sacchi et al. 2009).

Many lizard species are visually oriented and have a good colour vision (Loewe et al. 2002, Macedonia et al. 2009, Martin et al. 2014, reviewed in Olsson et al. 2013). Frequently, they display sexual colour dimorphism, in which adult males have bright colour patches. Furthermore, in many lizards colour patches may be used as a status badge (Rohwer 1975), when they signal RHP or dominance (Thompson and Moore 1991, Whiting et al. 2003, López et al. 2004, Stapley and Whiting 2006, Bajer et al. 2011, Hamilton et al. 2013). These patches are often located on the throat and ventrum, which make them only visible when they are intentionally displayed in the presence of a conspecific opponent, probably to reduce predation pressure (Martín and López 2001). However, there are also species that are colourful over the entire body (see Pianka and Vitt 2003). Overall, during male-male encounters, males' displays emphasise the colour patches, for example by extending the dewlap or lateral flattening of the body to present the ventral colour patch (e.g. Baird and Hews 2007, Macedonia et al. 2013), further supporting the idea that these colour patches are used as signals of RHP or dominance.

A variety of lizard species display multiple colour patches. According to the multiple messages hypothesis, different patches, but also different components of one colour patch (such as the chroma, size or number of patches), can indicate different quality aspects of the male (Møller and Pomiankowski 1993, Candolin 2003, Grether et al. 2004). For example, in the lizard *Lacerta schreiberi* the blue/UV head coloration and the green dorsum colour are more saturated in dominant males (Martín and López 2009). Whilst a more yellow chest patch with high UV reflectance signals a good immune response (Martín and López 2009). Alternatively, the redundancy hypothesis states that several colour patches may indicate the same information. By repeating information in different traits, it becomes easier and faster for conspecifics to assess the individual, and it makes cheating more difficult (Møller and Pomiankowski 1993, Candolin 2003, Szamado 2011). In lizards, females may evaluate multiple signals simultaneously when choosing a mate (Lancaster et al. 2009, Swierk et al. 2012). However, as far as we know, no research has been done to determine whether male lizards also use multiple signals in the assessment of a male opponent.

Dickerson’s collared lizard, *Crotaphytus dickersonae*, shows a strong sexual dimorphism with males being 14% longer and 63% heavier than females (Plasman et al. 2007). Males display a deep cobalt blue colour over their entire body, whereas females are brown with yellow legs and tail (McGuire 1996). Both sexes have a collar of two black bands, divided by a white stripe, and numerous white spots on the dorsum. This colouration makes the males, but not the females, very conspicuous in their habitat, the Sonoran desert. High detectability would be expected in territorial males where nearest neighbours are far away (Charles and Ord 2012), and in all studied *Crotaphytus* species males are very territorial with big territories that overlap the home ranges of several females (McGuire 1996). Detectability is further increased by behavioural displays, such as full show or extension of the gular area, that collared lizard males make at high perches and by which colouration is emphasised. These displays have been related to the intensity of aggression (Baird et al. 1997, Husak 2004, Baird 2013). Therefore it is likely that sexual selection has been involved in the evolution of the striking colour pattern displayed by these males. Accordingly, a recent study found that the blue dorsal colouration and the lightness and area of the black collar of the males were related with key morphological and performance traits associated to RHP (Plasman et al. 2015). Also, the lightness of the black collar was related with immune response, suggesting that the collar might also be used as a signal of condition (Plasman et al. 2015). In this study we evaluated whether males use colour traits to assess the opponents fighting capacity in male-to-male confrontations. First, we staged agonistic male encounters in which males differed in natural colouration. We predicted that males with higher blue chroma and bigger and darker black collars would receive less aggression. Secondly, we manipulated collar area to determine whether this colour patch would affect agonistic behaviour of the opponent males. We predicted that males with increased collar area would receive less aggression.

Methods

The study was conducted during the breeding periods of 2011 (June and July) and 2012 (May and June) of *C. dickersonae*. In total 40 males, 22 in 2011 and 18 in 2012, were captured in the hills between Bahía de Kino and Punta Chueca, Sonora, Mexico. Males were captured using a fishing pole with a noose and brought in individual cloth bags to the research station in Bahía de Kino. This location falls within the natural distribution of this species and thus allows for natural light-dark and temperature cycles. Males were kept in individual cages (60 x 60 x 60 cm) made of a wooden frame covered with mesh and fitted with canvas between adjacent cages and at the back of each cage to avoided visual contact between males, and reduce stress caused by passing people.

On the day of capture, after allowing the males to sunbath, colour measurements were taken with a spectrophotometer (MINOLTA CM-2600d; Minolta Co. Ltd, Osaka Japan) that determines reflectance from 360 to 740 nm at

10 nm intervals. Three measurements were taken of the blue dorsum colour, these were averaged, and blue chroma was calculated as the sum of the reflectance from 400 - 480 nm divided by the total reflectance. To estimate lightness of the collar, a measurement was taken from each of the black stripes of the collar. The two measurements were averaged and lightness of the black collar was calculated as the total sum of reflectance from 360 to 740 nm. During the 2011 trials we noted a pronounced change in colour in one of the males after a trial, therefore in following trials we measured colour traits of both males immediately before and after the trial (four trials in 2011, all trials in 2012). To estimate the black and white areas of the collar and the number of white spots on the dorsum males were placed on a white flat surface next to a ruler, necessary for scale, and a picture was taken with a camera Nikon Coolpix. Areas were measured with the histogram option in Adobe Photoshop (version 8.0). Additionally, snout vent length (SVL; ± 1 mm) and body mass (PESOLA scale; ± 0.1 g) were measured.

Behavioural trials

In the year 2011, 11 trials were performed to evaluate whether males use colour traits as signals of RHP. Males were paired as to have the greater natural differences in coloration in blue chroma, lightness and area of the collar, and number and area of white spots on the dorsum, while still similar in size (SVL: mean 89.00 ± 8.56 SD, average difference = 1.81 ± 0.98, paired t-test: t = 1.57, df = 10, P = 0.15), because in many lizard species size strongly affects RHP (e.g. Olsson 1992, López and Martín 2001, Sacchi et al. 2009).

In 2012, nine trials were performed. Within trials, males were paired based on size (mean SVL = 97.56 ± 3.53 mm; mean difference = 0.88 ± 0.60 mm; comparison of SVL between treatments paired t-test = -0.31, df = 8, P = 0.76). However, to evaluate the potential role of the size of the collar as a signal of RHP, the area of the collar was manipulated. Within a trial the size of the collar was increased for one of the males and decreased for the other male. Males in the increased treatment had their collar augmented by 40%; the black (mean ± SD: 5.20 ± 0.59 cm^2) and white (2.05 ± 0.36 cm^2) stripes were painted with a black and a white non-toxic paint, respectively. Males in the decreased treatment had the size of their collar decreased by 25% (using blue, green, white and black non-toxic paint; mean black area 2.90 ± 0.67 cm^2, white area = 1.15 ± 0.38 cm^2). Manipulated areas in the reduced collar treatment still had sizes within the natural size range of the collar in this species (natural range of black area: 1.22 - 4.89 cm^2, mean: 2.74 ± 0.83 cm^2; natural range of white collar area: 0.44 - 1.56 cm^2, mean: 0.77 ± 0.16 cm^2), but manipulated areas in the increased treatment were slightly bigger. The colour manipulation changed the lightness of the collar, lightness of the black collar after treatment was above the natural range in three males (range after manipulation: 16 - 206, natural range: 14 - 159), lightness of the white collar after treatment was higher than natural range in one male (range after manipulation: 637 - 2746, natural range: 523 - 2469), but the lightness of all other experimental subjects fell within natural range of lightness. Most of the paint was lost within the following day, probably due to extreme drying of the paint in the hot weather; however, males would completely lose the paint at their next skin shedding. After the trials had taken place males were returned to the place of capture.

Trials during 2011 and 2012 took place in an arena of 80 x 200 cm, with fibreglass walls with a height of 80 cm. The floor was made up of small grey stones, and on each extreme of the arena a bigger grey stone, with a height of 20 cm was placed to allow for a high perch. At the start of the trial the area was divided in two equal parts by placing a wooden board in the middle. After 20 min of acclimatization the wood was removed and the males could see each other, display, approach and fight. Trials lasted 20 min and were recorded with a video camera (Sony Digital 8 Handicam). Afterwards, the frequencies of aggressive and submissive behaviours were registered from the videos. Aggressive behaviours considered in our study were: fight (the male starts a fight in which both males roll wrestling over the ground), attack (fast approach), bite, superimposition (the male lies on top of the opponent), push ups (male flexes front legs), full show (male arc its back and extends the gular area), lateral display (male arcs its back and positions himself so its lateral side is directed to the other male), circling on the spot, gaping display (male opens the mouth wide and in the direction of the other male), head bobbing (quick movements of the head), and approach (approach of the other male in a direct line). Submissive behaviours included retreat, flee and submit (Baird et al. 1997). Also, we recorded the time each male spent on the big rocks. The rock can be considered a high perch, where dominant males can broadcast and scout for the presence of conspecifics (Baird 2013). Furthermore, dominant males are usually more active than subordinate males as they have to patrol their territory (Ruby 1984, Baird et al. 1997, Salvador et al. 2008). Hence, we recorded the time each male spent active (which includes walking, running and jumping through the arena, but time spent interacting with the other male was excluded). Time spent on the rock and active were considered as time allocated to dominant behaviour. When the animal spent time in the corner or crouching behind the rock it was probably intending to hide in the absence of refuges, and time spent in such a manner was considered time allocated to submissive behaviour. M. Plasman registered all data and intra observer reliability was high (R = 0.97).

Statistical analysis

Fights were rare, however based on the time a male displayed from a high perch (high rock) and the amount of time active the males were classified as either dominant or subordinate. Within a trial, one male normally spent a greater proportion of time on the rock (dominant males: 307 ± 332 sec, subordinate males: 16 ± 39 sec; Wilcoxon: V = 129, P < 0.001), while the other male spent most of the time in the corner or crouching behind the rock (dominant male 50 ± 76 sec, subordinate males 385 ± 426 sec; Wilcoxon: V = 3.5, P < 0.001). Time a male was active also differed between dominant and subordinate males (dominant: 111 ± 92 sec, subordinate: 75 ± 84 sec; Wilcoxon: V = 99, P = 0.002). Time Allocation was calculated as the time on the rock + time active – time in the corner – time hiding behind the rock. Further, Graded Agonistic Score was calculated following Baird et al. (1997), by weighing aggressive and subordinate behaviours and turning all the behaviours into one variable (table 1). Interestingly Graded Agonistic Score differed only with a trend between dominant and subordinate males (V = 20.5, P = 0.09; mean for dominant males = 10.65 ± 30.85, mean for subordinates = 0.75 ±

2.67), probably due to the low frequency of agonistic behaviour. To reduce variables to a single dominance measure per male Dominance Index was calculated as follows: Graded Agonistic Score and the Time Allocation were standardized, so the mean is 0 and the standard deviation is 1. Standardized Graded Agonistic Score and Time Allocation, as they now had the same units, were added to form the Dominance Index in which the Time Allocation and Graded Agonistic Score had the same weight. Dominance Index of dominant males was higher than that of subordinate males (2.06 ± 1.52; $t = -5.90$, $P < 0.001$, $df = 18$). In one trial from 2012 dominance could not be determined, and the trial was removed from further analyses.

General linear models (GLM) were performed separated for the dominant males and the subordinate males, using their Dominance Index as response variable.

Models initially included: differences in blue chroma, lightness and area of the black and white stripes of the collar. Differences in coloration were calculated as the value of the dominant male – the value of the subordinate male. The behaviour of the opponent was also included into the model; the opponents Dominance Index, Time Allocation, Graded Agonistic Score, or number of dominant or submissive displays were tested in separated models. Still, to evaluate differences between aggression and how males spent the time, separate models were performed for Time Allocation and Graded Agonistic Score. It was required to add 10 points to the Graded Agonistic Score of all individuals to make all scores positive, and this variable was then further analysed with generalized linear model with poisson distribution. In the model of Time Allocation of subordinate males, Time Allocation was calculated as time hiding + time crouching - time on the rock - time active. 200 was added to scores of all individuals to make all data points positive. The data was then analysed using generalized linear models with binomial negative distribution.

To evaluate the effect of the manipulation of collar size on status of the males, we performed general linear mixed models (GLMM) with trial as random factor. Models initially included treatment of opponent, behaviour of opponent (either dominance index, time allocation or graded agonistic score), and differences in blue chroma and lightness of the collar (either as measured immediately before or immediately after the trial).

Furthermore we evaluated whether male colouration related with aggressive or submissive behaviour displayed. We performed GLM on Graded Agonistic Score, initial blue chroma, black area of the collar, white area of the collar, number of white spots, SVL and behaviour of the opponent were included as covariables.

Best models were selected using Akaike's Information Criterion (AIC). When several models had similar AIC's (difference in AIC less than 2), the most parsimonious model was considered the best model. Because of low sample size and because data was not always normally distributed, it was always verified that residuals of the models were normally distributed. Averages ± standard deviation are given throughout the results section.

Results

2011 trials

Within a trial, dominant males had smaller white collars than subordinate males (paired t-test: $t = 2.46$, $P = 0.034$, $df = 10$; Fig 1). Blue chroma, black area of the collar, lightness of the black collar, and number and area of white spots were not significantly different between dominant and subordinate males (paired t-test: $t < 1.46$, $P > 0.17$).

Dominant males

The Graded Agonistic Score of dominant males was related to the number of subordinate displays by the opponent and the difference in blue chroma (table 2). Dominant males displayed more aggressive behaviours when their opponents were bluer than they were (fig 2). However, other colour variables were unrelated to the Dominance Index and the Time allocation of dominant males (table 2).

Subordinate males

Subordinate males had higher (closer to cero) Dominance Indices when their white collars were similar or smaller than those of their opponents (table 3, fig 3), probably because Time Allocation was higher when their white collars were relatively smaller. Graded Agonistic Score was higher when the subordinate male had lower blue chroma than the dominant male, although this was not significant (P = 0.18), and was higher when he received more aggression (table 3).

Male colour change during a trial

Blue chroma did not change during the trial in dominant males (average change = 0.017 ± 0.023). However, subordinate males always lowered their blue chroma during the trial (average change = -0.026 ± 0.043), but their change in colour did not significantly differ from the change in colour of dominant males (t = 1.25, P = 0.30, n = 4). Still, males that displayed more subordinate behaviour reduced more their blue chroma (R = -0.81, P = 0.02, n = 4).

2012 trials (increased and decreased collar area)

General linear mixed models showed that treatment had no affect on the Dominance Index of the males or their Graded Agonistic Scores. Dominance Index and Graded Agonistic Score could only be explained by blue chroma, however this was not significant (t = 0.05, t = -0.82 resp.). Treatment did affect Time Allocation (t = 1.17; estimate = 285.79 ± 243.66). Further, when blue chroma (t = -0.20) and lightness were higher (t = -0.80) Time Allocation was lower.

Dominant males:

The model that best explained the Dominance Index of the dominant male was influenced by treatment, but the effect was weak and not significant (t = -1.67, P = 0.17). Dominance Index was higher when the opponent displayed more subordinate behaviour and when the male had lower blue chroma compared to its opponent (Table 4). Also, Time Allocation of the male was higher when the male had lower blue chroma than its opponent (t = -2.30, P = 0.06). Its Graded Agonistic Score did not relate to any of our measurements.

Subordinate males:

The Dominant Index of the subordinate males was lower when the opponent male had a decreased collar area (t = -3.78, P = 0.02; Table 5, Fig 5). Further, the Dominance Index of the subordinates was lower when the dominant male performed more dominant acts, and when the subordinate male had relatively high blue chroma (table 5, fig 6). Graded Agonistic Scores deviated only in two trials from 0 (-2 and -3) and was not further analyzed. Subordinates spent more time crouching or hiding when their opponent had reduced collars, had lower blue chroma, or higher Time Allocation (Table 5).

Colour relates to behaviour:

Males that presented a higher blue chroma immediately before the trial, were less active (R= -0.49, P = 0.04, n = 18), but had higher Graded Agonistic Scores (R = 0.48, P = 0.046). Males with lower blue chroma immediately before the trial displayed more subordinate behaviours (R = -0.53, P = 0.02). Males with more white spots spent more time crouching in a corner (R = 0.63, P = 0.005).

Colour change:

Males that reduced their blue chroma during trials tended to be those males that fled more (R = 0.47, P = 0.05). Change in blue chroma during the trial was correlated with a change in body temperature (R = 0.52, P = 0.03). Interestingly, males with increased collar size lowered more their body temperature (-1.96 ± 1.41 Cº) than males with reduced collar size (-0.05 ± 2.16 ºC; paired t-test: t = 2.53, df = 8, P = 0.04).

Colour change in subordinate males correlated with the Graded Aggression Score and the Dominance Index of the dominant opponent (R = 0.74, P = 0.02; R = 0.68, P = 0.044 respectively). Subordinate males that reduced more their blue chroma received less aggression (fig 7). Change in blue chroma in dominant males did not correlate to any of the behavioural variables.

Discussion

Conspicuous coloration is common in lizards (Cooper and Greenberg 1992). Bright or black colour patches are often used as status badges (reviewed in Whiting et al. 2003, Olsson et al. 2013), in which more intense colours or bigger colour patches may signal winning chance (Olsson 1994, Whiting et al. 2006, Bajer et al. 2011, Henningsen and Irschick 2012). In an earlier study it was found that the black collar and the blue chroma of male collared lizards were related to performance measurements that may indicate recourse-holding power (Plasman et al. 2015). In this study we evaluated whether males use these colours as signals to estimate the opponent's fighting ability.

We found that neither black collar area, nor blue chroma determined the outcome in our male-competition trials. However, males that were bluer before the trial were more aggressive, and dominant males were more aggressive towards bluer opponents. When colour is used as a status badge it is expected to reduce aggression (Rohwer 1975), but we found that males with higher blue chroma received more aggression. Yet blue colouration could change in short time spans. Males that displayed more subordinate behaviours reduced more their blue chroma. Moreover we found that males that reduced more their blue chroma during the trial, received less aggression. Hence, blue chroma may be a dynamic signal of dominance or rather a lowered blue chroma may signal subordinance.

Colour changes in lizards after intra-sexual encounters have been observed in other species. The most spectacular example comes from *Anolis carolinensis*, in which males, after losing a fight, change body colour from green to brown and display more submissive behaviour (Greenberg and Crews 1990). A change in male status badge or behaviour due to previous fighting experience will affect their subsequent winning chance and is called the winner-loser effect. This effect causes males that win a fight to have higher winning chances in subsequent fights, whereas losers in a first fight tend to lose later fights (Rutte et al. 2006). In the lizard *Ctenophorus vadnappa* male winning chance initially relates to colour, however in following contests experience is more important (Stuart-Fox and Johnstone 2005). We do not know prior fighting experience of the males used in this study, or even if they had established territories. However, our data suggest that the blue colouration of Dickerson's collared lizard is a dynamic signal that alters the levels of aggression received and is associated with subordinate behaviour. Hence a decrease in blue chroma may be considered a loser-effect.

Interestingly we found that, at least when collar size was not manipulated, white collars were bigger in subordinate males, and subordinates that had relatively big white collars spent more time crouching, even though white collar size was positively related with bite force (Plasman et al. 2015). Also, when collar area was manipulated, subordinates displayed more subordinate behaviour when their opponent had decreased collar area, although both black and white collar areas were adjusted and smaller black collar area should indicate lower RHP (Plasman et al. 2015). Hence, the white collar may signal information other than performance, which could be more decisive for the outcome of male-male competition. Age, for example, influences fighting experience and often older males are more dominant (Smith 1985, Aragón et al. 2004, Wikelski et al. 2005). White collar area was however not related with body size (at least not directly, see Plasman et al. 2015), which is likely to indicate age in a species with indeterminate growth (Sexton 1992). Still, within a size-class smaller collars could indicate age.

Collar size might also signal reproductive strategy. Reproductive strategy may be signalled by different morph colours, especially when the strategy is fixed (Sinervo and Lively 1996, Hews et al. 1997). However when the strategy is plastic and animals can change between strategies depending on condition or social environment, more subtle signals may be used. Whiting et al (2006) found that floater males had lower UV chroma in the throat colour patch than territorial males. Collared lizards males may either be territorial or floaters (Baird and Timanus 1996, Faber et al. in prep.). In the present study we do not have knowledge about age, experience or reproductive strategy of the males, however the subordinate behaviour of males with big white collars may indicate that they are less experienced fighters.

Another interesting fact was that the manipulation of collar area affected body temperature. Males that had an increased collar lowered more their body temperature. Males with naturally bigger collars also have lower body temperatures and collar area may signal the ability to maintain low body temperatures at high environmental temperatures (Plasman et al. 2015). Yet when collar area is manipulated, this should not affect the male's physiology. Moreover, bigger black collars should increase heat absorption (Clusella-Trullas et al. 2007). Change in body temperature was positively related with a change in blue chroma. Blue coloration relates to body temperatures in several species (Morrison et al. 1996, Hettyey et al. 2009, Langkilde and Boronow 2012), including Dickerson's collared lizard (Plasman et al. 2015). Hence, temperature may be the mechanism by which blue colouration is adjusted.

In the present study we found low levels of aggression, which may indicate that the males use quick, easily evaluated signals, like visual signals, to avoid direct confrontations. We found that collar and blue dorsal colour did not determine dominance, but they did affect the level of aggression. Possibly several signals acting together as a pattern, rather than single colour traits, determine dominance. Furthermore, conspicuous colours can be important from a distance (Losos 1985, Whiting et al. 2003), whereas nearby other aspects of the male’s morphology are assessed. Also, experience and motive are likely to affect the outcome of male-male interactions (Schwartz et al. 2007; Sacchi et al. 2009). Due to lack of resources in the fighting arena, motivation to fight was probably low and it would be exciting to evaluate male-male competition in the field. Interestingly we found that male blue dorsal colouration could change during the trials, and sometimes changes were spectacular (fig 8). Although the dynamics of colour change, the information it signals, and how long its effects last should be further investigated, our results suggest that the blue colour could be a dynamic signal of current dominance status.

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Tables

Table 1. Weights of behaviours, observed during the male contest trials, used to calculate graded agonistic behaviour score.

Behaviour	
Bite	+3
Fight	+3
Attack	+3
Superimposition	+3
Display	+2
Circle	+2
Push-up	+2
Full show	+2
Head bobbing	+2
Gape	+2
Approach	+1
Retreat	-1
Flee	-1

Table 2. Behaviour of the dominant male considering opponent's behaviour and difference in natural colouration. The best models are given for Dominance Index and Graded Agonistic Score. Time allocation did not relate with any of the measured behaviours or colour traits. Dominance Index is explained by the number of subordinate displays of the opponent, whereas Graded Agonistic Score also depended on the difference in blue chroma between the males.

Dominance Index	estimate	95%CI (lower, upper)	t	P
Subordinate displays	0.18	(0.06, 0.30)	2.98	0.02
Graded Agonistic Score				
Subordinate displays	0.17	(0.12, 0.23)	6.20	<0.01
Blue chroma	8.34	(0.55, 16.12)	2.10	0.04

Table 3. Behaviour of the subordinate male considering opponent's behaviour and differences in natural colouration. The best models are given for Dominance Index, Graded Agonistic Score and Time Allocation.

Dominance Index	estimate	95%CI (lower, upper)	t	P
White collar area	2.66	(1.03, 4.29)	3.20	0.01
Graded Agonistic Score				
Aggressive displays	0.19	(0.04, 0.34)	2.53	0.04
Blue chroma	45.10	(-14.54, 104.74)	1.48	0.18
Time Allocation				
White collar area	1475.50	(620.16, 2330.84)	3.38	0.01

Table 4. Behaviour of the dominant male in trials in which collar area was manipulated to be either big or small. The best models are given for Dominance Index, Graded Agonistic Score and Time Allocation.

Dominance Index	estimate	95%CI (lower, upper)	t	P
Treatment	-0.38	(-0.83, 0.07)	1.67	0.17
Subordinate displays	1.01	(0.81, 1.21)	9.70	<0.01
Blue chroma (after trial)	-22.30	(-29.09, -15.51)	-6.43	<0.01
Graded Agonistic Score				
Treatment	-0.02	(-0.43, 0.39)	-0.11	0.92
Time Allocation				
Treatment	-0.05	(-1.08, 0.99)	-0.04	0.97
Blue chroma (after trial)	-13.77	(-29.06,1.52)	-1.77	0.08

Table 5. Behaviour of the subordinate male in trials in which collar area was manipulated to be either big or small. The best models for the Dominance Index and Time Allocation are given.

Dominance Index	estimate	95%CI (lower, upper)	t	P
Treatment	1.40	(0.68, 2.13)	-3.78	0.02
Aggressive displays	-0.54	(-0.89, -0.18)	-2.95	0.04
Blue chroma (before trial)	21.00	(7.87, 34.12)	3.14	0.04
Time Allocation				
Treatment	-1.21	(-1.57, -0.86)	-6.88	<0.01
Time Allocation	0.002	(-0.002, -0.001)	-4.83	<0.01
Blue chroma (before trial)	-29.11	(-34.49, -23.73)	-10.61	<0.01

Figures

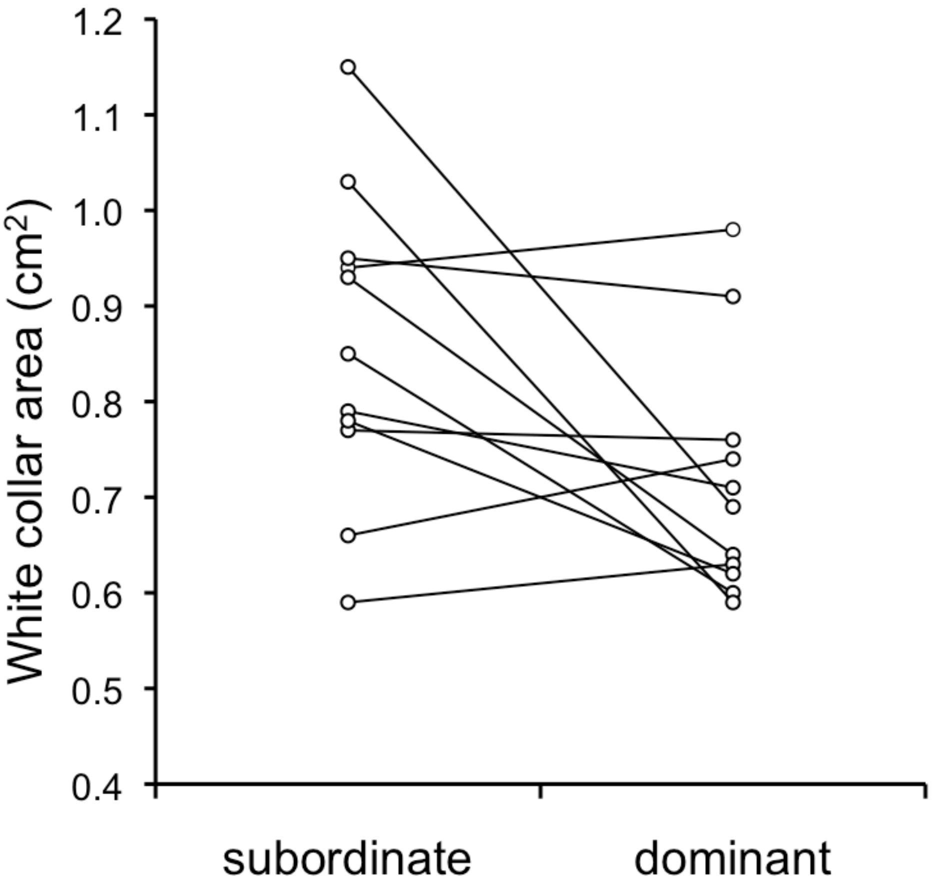

Figure 1. The white area of the collar (cm^2) of the subordinate and dominant males. Lines connect the subordinate and dominant male from the same trial.

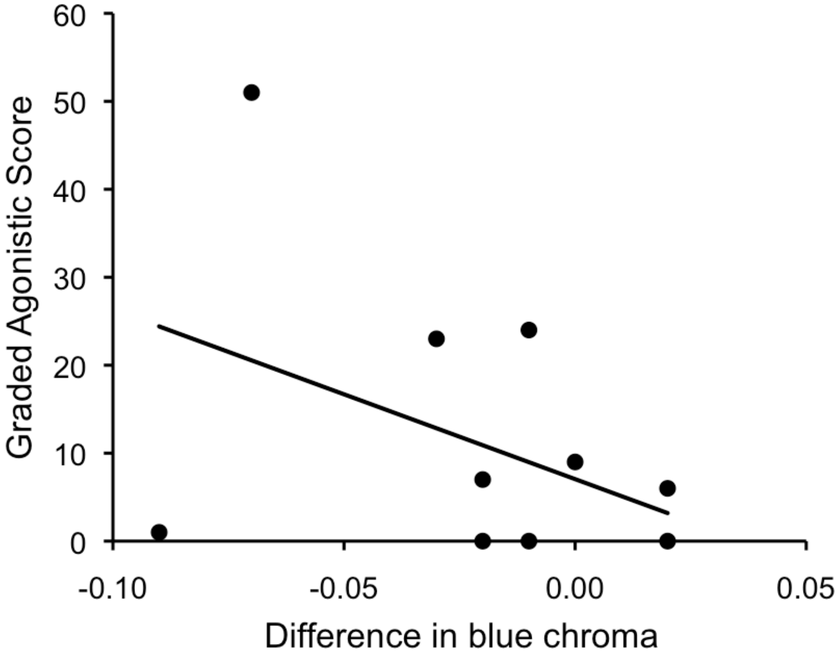

Figure 2. The relation between graded agonistic score of the dominant male and the difference in dorsal blue chroma between the males. Negative blue chroma difference indicates that the dominant male had lower blue chroma than the subordinate male.

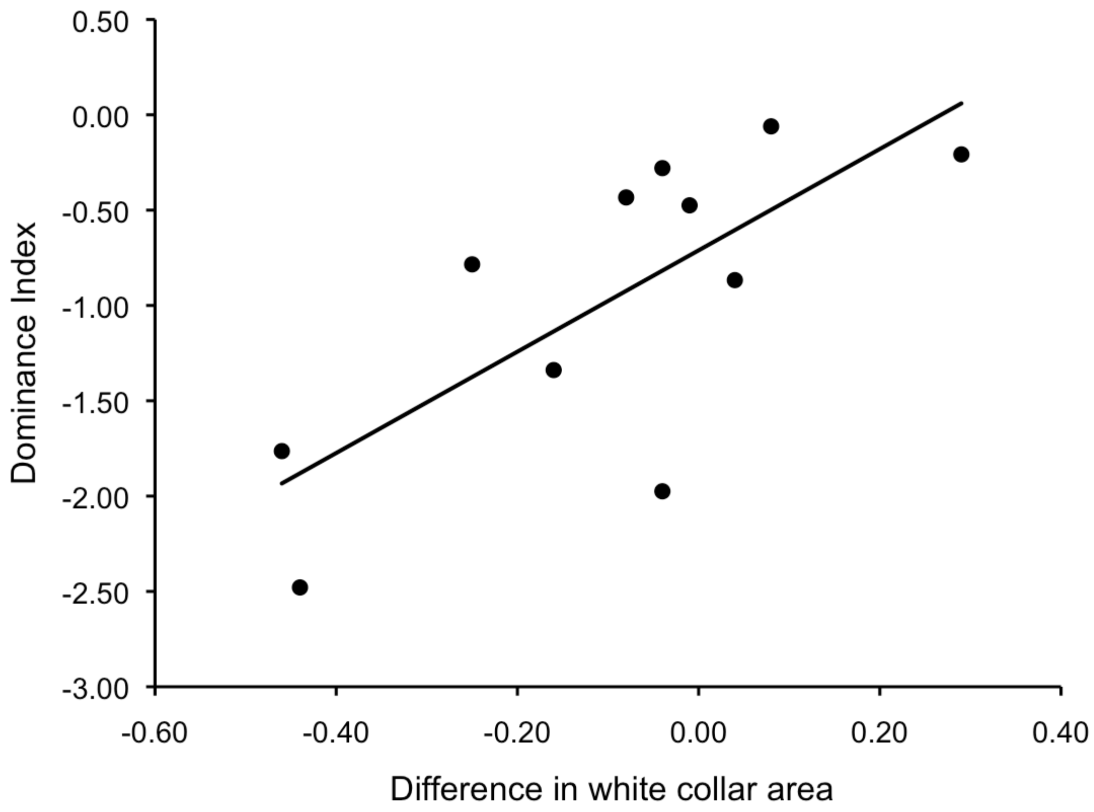

Figure 3. Dominance Index of the subordinate males related with the difference in white collars. A negative Dominance Index indicates more submissive behaviours were observed. A negative difference in white collar area indicates that the subordinate male had a bigger white collar than the dominant male.

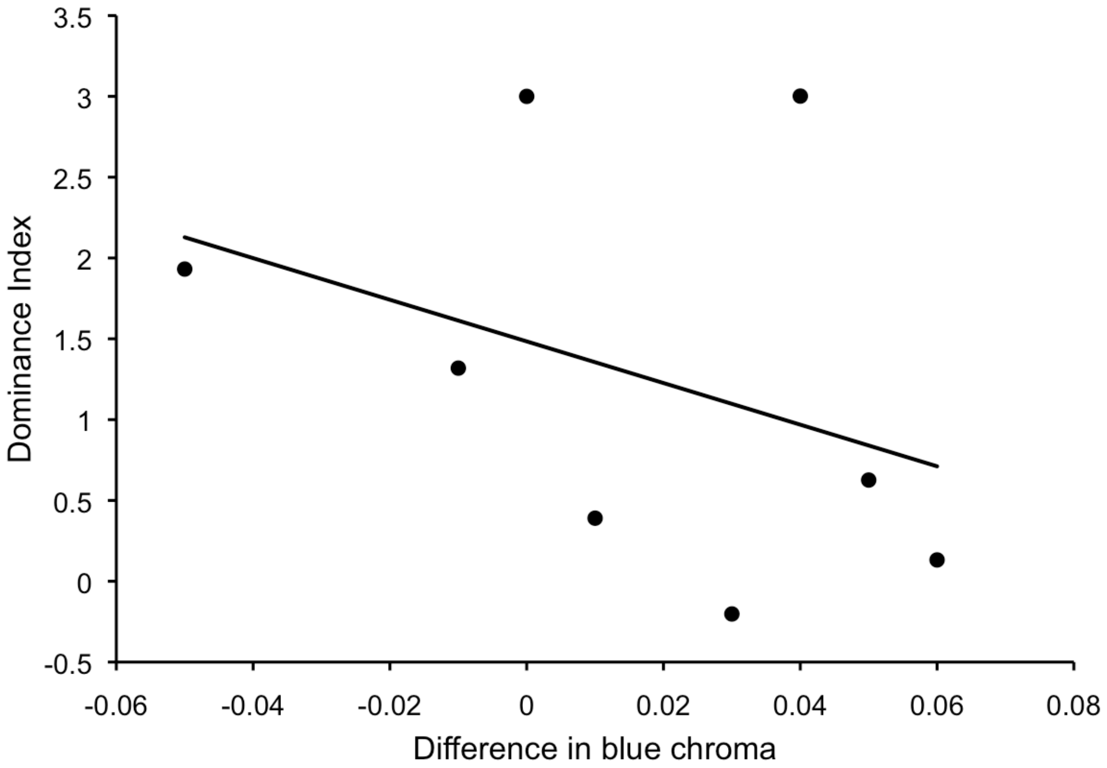

Figure 4. The Dominance Index of the dominant male was higher when subordinate males were bluer. Negative Dominance Index indicates more subordinate behaviours than dominant behaviours. Negative difference in blue chroma indicates that the subordinate male had higher blue chroma than the dominant male.

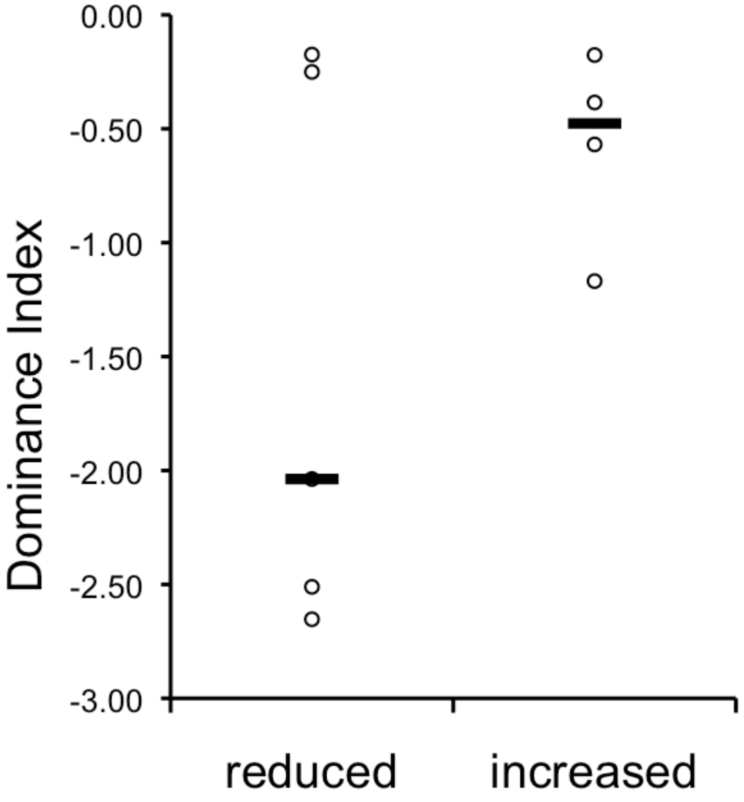

Figure 5. The Dominance Index of the subordinate males when their opponents had reduced collars (n = 5) or increased collars (n = 4). Circles indicate Dominance Index recorded and horizontal bars represent the average Dominance Index of the subordinate males (graph design taken from Weissgerber et al. 2003).

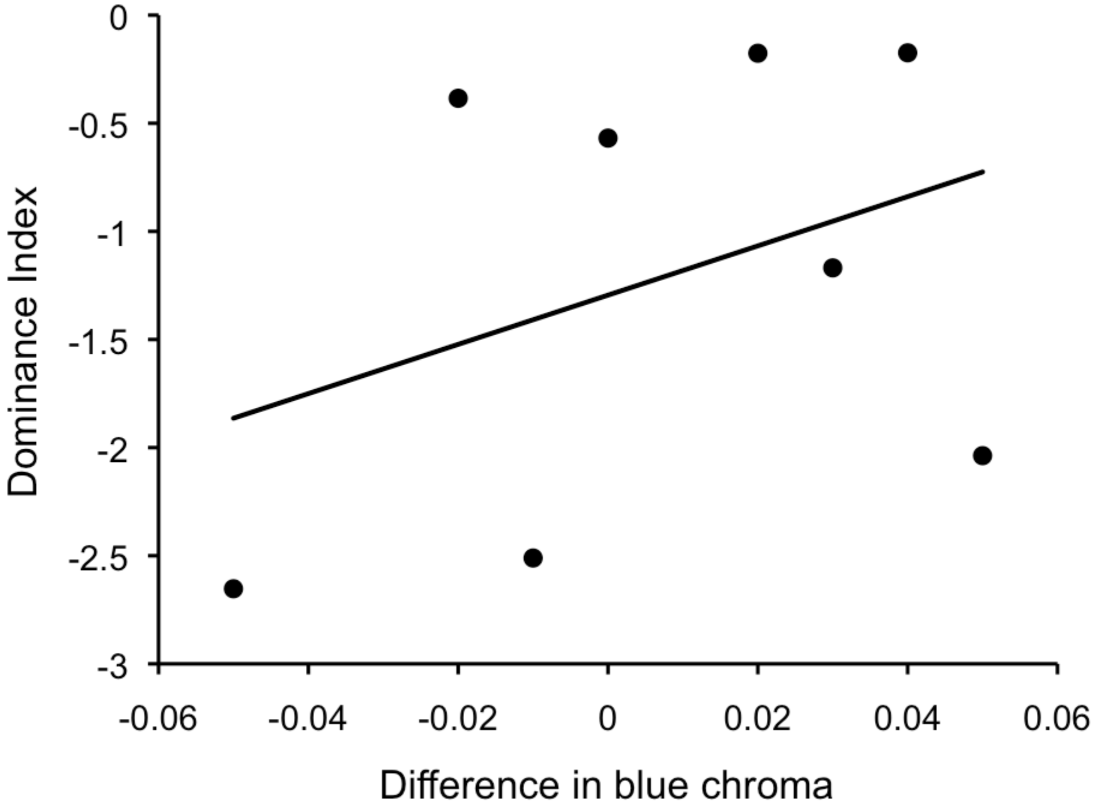

Figure 6. The Dominance Index of the subordinate male (in the trials in which collar area was manipulated) depended on the difference in blue chroma at the beginning of the trial. Negative Dominance Indices indicate more submissive behaviours than aggressive behaviours. Negative differences in blue chroma indicate that the subordinate male had higher blue chroma than the dominant male.

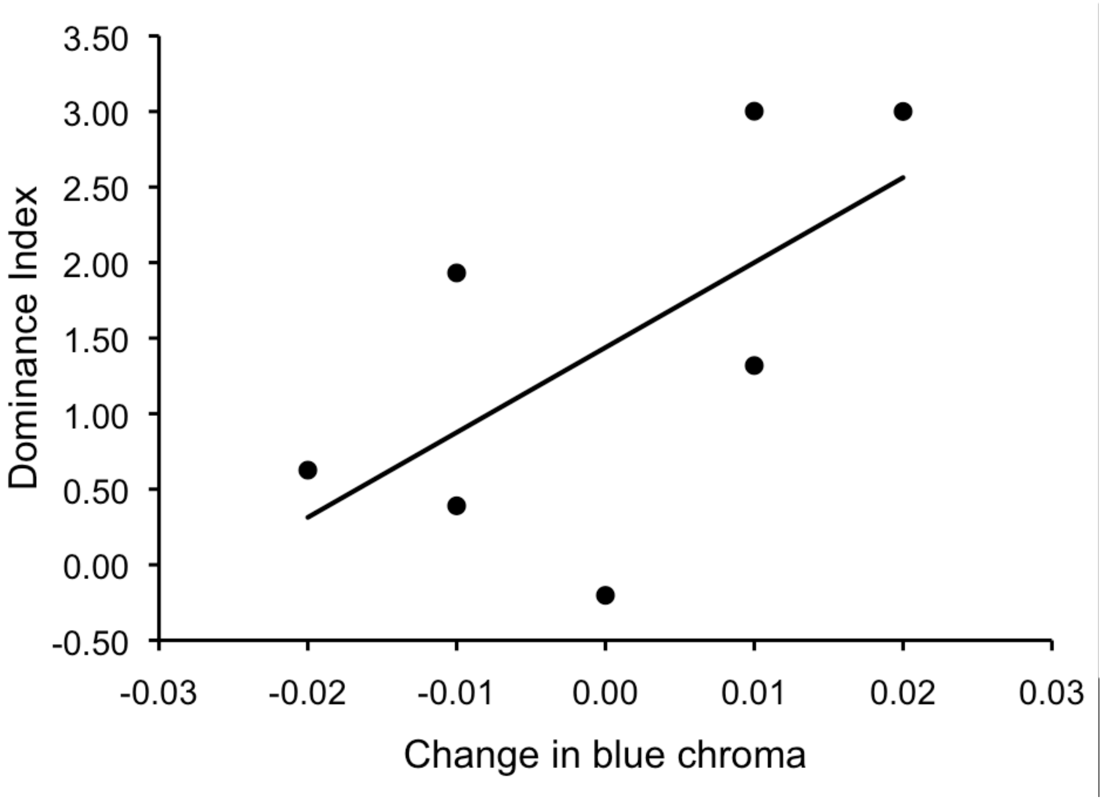

Figure 7. The relation between the dominance index of the dominant male and the change in blue chroma during the trial of the subordinate male. Negative dominance Index indicates more submissive behaviours than dominant behaviours. A negative change in blue chroma indicates that the male reduced its blue chroma during the trial.

Figure 8. Strong colour change in Dickerson's collared lizard. The three pictures show the same male, to the left just before the male competition trial, in the middle immediately after the trial and to the right the male after release. Please note that in the last picture light circumstances are different.

Capítulo 5.

Feeling the heat: Compromises at extreme temperatures between immune response and skin colour in a desert dwelling lizard

Chapter 5: Feeling the heat: Compromises at extreme temperatures between immune response and skin colour in a desert dwelling lizard

Melissa Plasman and Roxana Torres

Abstract

In ectotherms, temperature affects performance, physiology, and in some species, skin colour. Skin colour might consequently be a dynamic signal of condition, particularly in habitats with extreme temperature fluctuations, as animals have to maintain body temperatures that diverge from environmental temperatures. Dickerson's collared lizard, *Crotaphytus dickersonae*, males are blue and have a black and white collar, this conspicuous colouration signals performance and immune response. By maintaining males at higher, lower, and mean environmental temperatures we evaluated whether ambient temperature influences colour and immune response (estimated through hemolysis-hemagglutination assays), and whether at extreme temperatures there is a trade-off between colour and immune response. We found that males' blue colouration and immune response were reduced at extreme temperatures (i.e. high and low temperature treatments). At low temperatures, big males that showed a weaker immune response also decreased blue chroma and became greener. At high temperatures however, small males that showed a weaker immune response maintained a bluer skin colour. The results suggest that blue colour of Dickerson's collared lizards is a dynamic signal that at high temperatures may compromise the immune function.

Keywords: sexual colour, colour change, dynamic signal, trade-off, ecophysiology

Introduction

Understanding how trade-offs between natural and sexual selection may shape life histories is of great interest for evolutionary biologists. One of these trade-offs is the one between the production of colourful sexual signals and the immune system*.* Sexual signals are important for reproduction because they facilitate intra-specific communication by reducing costs associated to evaluating possible mates or competitors (Andersson 1994). The immune system, on the other hand, is essential for survival and it has been considered one of the most important determinants of lifetime fitness (Lochmiller and Deerenberg 2000). However both, the production and maintenance of conspicuous sexual traits and the immune function are expected to be costly (reviewed in Lochmiller and Deerenberg 2000, Hill 2011, Tibbetts 2014) and resources will have to be divided between those traits.

Sexual traits and the immune system can be linked either because they share pathways, or a metabolite stimulates one trait but restrains the other (Hill 2011). Testosterone, for example, is often involved in male sexual traits, including colour, but may have immune repressor effects (Folstad and Karter 1992). Further, an allocation trade-off exists in several species in which carotenoids either function as pigments or antioxidants (Lozano 1994, McGraw 2005). Melanocytes, the melanin carrying pigment cells, have phagocytic abilities, can present antigens, neutralize toxins of bacteria, and capture free radicals (reviewed in Macintosh 2001, McGraw 2003, 2005). Melanogenesis is often stimulated locally at the place of injury or infection (Lévesque et al. 2013) and genes involved in melanogenesis are implicated in other physiological processes including the immune system (Ducrest et al. 2008). Melanin based colours are positively related with the immune response in some species (e.g. Griffith et al. 2006, Gasparini et al. 2009). Structural colours are caused by platelets called iridophores that reflect light of certain wavelengths. Like the immune system, iridophores might be affected by vitamin A (San José et al. 2013). In some species, structural colours have been related with immune response and parasite load (e.g. Shawkey et al. 2007, Griggio et al. 2010). Yet the relation between structural colours and the immune system has been rarely studied.

The fact that ectotherms depend on environmental temperature to determine their body temperature has been a driver and a constraint in their evolutionary history. Body temperature of ectotherms affects level of physical and physiological performance, influencing a variety of traits such as locomotor performance, growth, metabolic rate, fecundity (see Angilletta et al. 2002, Angilletta 2009 and references therein), immune response (eg. Zimmerman et al. 2010), and colour (Langkilde and Boronow 2012). Generally, there is an optimal body temperature at which performance level is highest, and at lower and higher temperatures performance drops (Angilletta et al. 2002, Angilletta 2009). Individuals may differ in their capacity to maintain optimal body temperatures with varying environmental temperatures, for example due to body condition (e.g. Seebacher 2005). Moreover, structural differences in enzymes can lead to different optimal temperatures and differences in sensitivity within a species (Angilletta 2009).

The vertebrate immune system is complex and involves many pathways (Bona 2005). Nevertheless when assessed in ectotherm vertebrates, the immune response always seems to decline at extreme temperatures, whether due to *in vitro* incubation temperature (*eg.* Sypek et al. 1984, Bly and Clem 1992, Maniero and Carey 1997, Le Morvan et al. 1998, Merchant and Britton 2006, Palacios et al. 2013, Merchant et al. 2014; but see Butler et al. 2013) or to acclimation temperatures (e.g. Bly and Clem 1992, Maniero and Carey 1997, Le Morvan et al. 1998, Jackson and Tinsley 2002, Rollins-Smith and Woodhams 2011). For example, in the lizard *Hemidactylus flaviviridis*, *in vitro* incubation temperature affected phagocytic ability with lower performance at low (7 and 15ºC) and high (37ºC) temperatures compared to intermediate temperatures (25ºC; Mondal and Rai 2001). Also, acclimation temperature affected the response *in vivo* to a bacterial antigen (lipopolysaccharide, LPS) by *Iguana iguana* (Deen and Hutchinson 2001). Iguanas kept at high temperatures were more likely to respond by behavioural hypothermia, instead of fever, than animals kept at lower temperatures. Interestingly, iguanas that induced a fever had higher body mass, which might indicate that the energy reserves of the animal affect their immune response.

Body temperature may also affect colour traits. Ectotherms are often darker in cold climates. This adaptation allows for shorter time to heat up and thus permits a longer activity period (Clusella-Trullas et al. 2007). However, at high temperatures, dark colours can lead to overheating and some animals have developed the ability to darken when cold and to lighten their colours at high temperatures (Sherbrooke 1997). Darkening is caused by the dispersal of melanophores within the pigment cells called melanocytes (Bagnara et al. 1968, Tonosaki et al. 2004). This physiological colour change is quick (with maximal colour change in less than two hours) and can be reversed (e.g. Duellman and Trueb 1994).

Structural colours can also be affected by temperature and could change in short periods of time (<1h; Langkilde and Boronow 2012). Structural skin colouration results from the reflectance of light from stacked platelets in pigment cells (iridophores) that depending on the size, spacing, and angle of these platelets will reflect certain wavelengths, usually in the UV or blue range (Morrison et al. 1996). Temperature can alter the spacing of the platelets and thus the wavelengths reflected by the skin (Morrison et al. 1996). Bluer male frogs (*Rana arvalis*) and European green lizards (*Lacerta viridis)* with higher UV reflectance in their nuptial throat patches were individuals with higher body temperatures (Hettyey et al. 2009, Bajer et al. 2012). Moreover, males of the Eastern fence lizard (*Sceloporus undulates)* change throat and ventral colouration from green to blue when body temperature increases (Langkilde and Boronow 2012). Interestingly, in all of these examples the colour traits dependent on temperature are sexual signals: in the frog and the green lizards the colour patch is used to attract females (Hettyey et al. 2009, Bajer et al. 2010) and in the fence lizard it indicates status (Langkilde and Boronow 2012).

Dickerson's collared lizard (*Crotaphytus dickersonae*) males are bright blue, whereas females are dull brown with yellow legs and tail (McGuire 1996; Macedonia et al. 2009). Both sexes have a collar composed by two black stripes divided by a white one. The conspicuous male colouration is most likely involved in sexual selection. Males with a bluer dorsum and darker and bigger black collars had greater scores on traits related to resource-holding power (Plasman et al. 2015). Further, males with a darker collar had fewer parasites and responded with more inflammation to an antigen (phytohemagglutanin), indicating strong immune responses (Plasman et al. 2015). Interestingly, males with high body temperatures were bluer, and their black collar bands were smaller and of a lighter black coloration (Plasman et al. 2015). Inflammation response to phytohemagglutanin was also strongly and positively related to body temperature (Plasman et al. 2015). Hence, it is likely that in males of this species, coloration is a dynamic signal that might fluctuate in synchronization with the immune response. Yet, as both immune response and sexual colour traits are probably costly (Lochmiller and Deerenberg 2000), males may face a compromise between displaying a colour indicating high quality and dealing with possible costs of a decreased immune response, or they may have to reduce sexual colouration to maintain an optimal immune function. When the trade-off is modulated by temperature, extreme temperatures may expose these trade-offs (Lazzaro and Little 2009).

To investigate the influence of temperature on the potential trade-off between sexual colour display and immune function we experimentally manipulated the environmental temperature at which males were exposed. We predicted that immune response and colour would be lower at extreme temperatures compared to control. Further, trade-offs may be observed in the extreme temperature treatments in which males invest in either the sexual colour or the immune response. Additionally, we expected that males of better condition (larger and heavier males) and males with lower parasite load would be better able to withstand the costs of temperature stress and be able to maintain both colour and immune response, whereas low quality males (smaller, lighter males with higher parasite load) would reduce colour and immune response at extreme temperatures and would be more likely to have to compromise between these two traits.

Methods

The study was conducted from May to June 2012 in Bahía de Kino in the state of Sonora, Mexico. During the reproductive period of Dickerson's collared lizard, 18 adult males were captured between Bahía de Kino and Punta Chueca, and brought in individual bags to a research station in Bahía de Kino, which is located within the distribution range of this species and therefore lizards experienced similar lighting, temperature, and humidity as in their natural habitat. Males were kept in individual outdoor cages (60 x 60 x 60 cm) made of a wooden frame covered with mesh. Canvas between adjacent cages and at the back of each cage avoided visual contact between males and reduced stress caused by passing people. Every morning individuals were fed with one or two live crickets, water was provided *ad libitum*, and cages were misted.

On the day of capture the male’s snout-vent length (± 1mm; hereafter SVL) and body mass (± 0.1 g; PESOLA scale) were measured. Different components of the immune system interact (Demas et al. 2011), and because parasite load can affect immune response (Goldberg and Holshuh 1992, Huyghe et al. 2010, Plasman et al. 2015), we collected and counted all ectoparasites found on the body of each male and on the bags in which the males were transported on the day of capture.

Temperature treatments

To assess the effect of environmental temperature on the males’ colour traits and immune response, each male endured three different environmental temperature treatments consecutively, each lasting 48 hours at which moment measurements were taken and the male was moved to another treatment (n = 18). The order in which males entered the treatments was determined at random. In the low temperature treatment, cages were placed in the shade and to reduce light differences with cages from other treatments, a cold white light (a neon light bulb) was place in each cage. In the control treatment, cages were directed towards the west. In the high temperature treatment, cages were oriented towards the sun. All cages were fitted with a refuge to reduce stress and to offer an escape from lethal temperatures. Per treatment, two dataloggers (Hobo Temperature Datalogger H02-001-02) that measured environmental temperature every 30 min were placed in separate cages. Within a treatment, temperatures measured at the same time in two different cages were similar (Pearson correlation: $R > 0.97$) and repeatability between days was high (covariance for low temperature treatment = 6.44, control = 11.83, high temperature = 11.83).

In the coastal area of the state of Sonora, Mexico, the natural habitat of our subject species, the average temperature was 25-30ºC for May 2012 and 30-35ºC for June 2012 (smn.cna.gob.mx). The average temperature in our control treatment was 30.97 ± 0.89ºC, and thus within the natural range. In the low temperature treatment the males experienced significant lower temperatures, with an average of 25.80 ± 1.47ºC (repeated measures ANOVA: $F_{2,41} = 27.86$, $P < 0.01$; paired t-test: $n = 18$, $P < 0.01$). Lowest average daily temperature in May and August observed in Bahía de Kino is 24ºC (smn.cna.gob.mx) and thus our low temperature treatment falls within natural range. Average temperature did, however, not differ between control (30.97 ± 0.89ºC) and high temperature treatment (30.13 ± 0.98ºC; paired t-test: $P = 0.11$). Yet maximum temperatures in the high temperature treatment (60.64 ± 2.34 ºC) were in average 5ºC higher than those of the control (55.32 ± 3.65ºC), nevertheless the difference was not significant (paired t-test: $P = 0.08$). Still, even slight increases in temperature (~1ºC) can have strong biological consequences (e.g. Garvin et al. 2006, Angilletta 2009, Dillon et al. 2010) and males in this experiment responded differently to the control and high temperature treatment for which we included both control and high temperature treatments in the analyses. Also, minimum temperatures differed between all treatments (repeated measures ANOVA: $F_{2,41} = 7.04$, $P = 0.002$; paired t-test: $P < 0.04$). Interestingly mean minimum temperatures were highest in the low temperature treatment (22.13 ± 1.59ºC), compared to the control (20.27 ± 0.39ºC), and minimum temperatures were lowest in the high temperature treatments (17.10 ± 1.88 ºC). Hence, at the high temperature treatment males experience stronger temperature fluctuations during the day, which may increase temperature stress (Vasseur et al. 2014).

To evaluate the effect of the temperature treatment on the males, on the second day of each treatment we took a blood sample for later immune response analysis. The blood sample (100 microL) was taken from the caudal vein in the tail. Blood samples were centrifuged during 10 min at 6000 rpm to separate plasma from the cellular fraction and plasma was stored in liquid nitrogen and later in a deep-freezer (-80ºC) until immune analyses were performed. Immediately after blood sampling, cloacal temperature (± 0.1 °C; Miller and Weber, Inc), an indicator of body temperature, and colour were measured. This procedure was conducted between 13:00 and 14:00 hours. After these measurements the male was moved to the next treatment.

Colour measurements

To evaluate the effect of environmental temperature on the male colour traits, colour measurements of the blue dorsum and the black collar were taken with a portable spectrophotometer that determines reflectance from 360 to 740 nm at 10 nm intervals (Minolta CM-2600d, Osaka Japan). For each male, we took three measurements of the blue dorsal colour and from the mean reflectance curve of these measurements we calculated the blue chroma as the sum of reflectances from 400 to 480 divided by the sum of total reflectance (Plasman et al. 2015). Males with higher blue chroma are deeper cobalt blue than those with lower blue chroma. From the collar, one measurement from each of the two black bands was taken and the lightness of the black collar bands was calculated as the sum of total reflectances from 360 to 740 nm from the mean reflectance curve of both black bands. High lightness indicates a relative light black collar and low lightness a darker black collar. Photographs from the lateral and dorsal sides of each male were taken to determine the number of the white spots and estimate the area of the collar by counting the number of pixels per colour using the histogram option of Adobe Photoshop (version 8.0). Males were placed on a flat white surface with a ruler as scale reference and pictures were taken from 40 cm distance with a Nikon Coolpix camera.

Immune response

Immune response was estimated by a hemolysis/hemoagglutination assay as described by Matson *et al*. (2005). This assay is based on two components of the humoral immune response: natural antibodies (NAbs) and complement pathway. Briefly, NAbs opsonise invading micro-organisms: they mark the micro-organisms for destruction by phagocytes, and trigger lysis by stimulating the complement pathway. The concentration of NAbs and B-1 cells determines whether agglutination and subsequently lysis occurs, or agglutination happens but not lysis, or whether microorganisms are not affected (Matson et al. 2005).

To simulate, *in vitro*, an invading microorganism we used rabbit whole blood conserved in Alsever (Dibico 1602 FC). Every day the quantity of rabbit blood necessary was washed: the blood was centrifuged 2000 rpm/ 5 min and the supernatant was discharged to remove the Alsever. Then, phosphate buffered saline solution (PBS) was added, the suspension was centrifuged (2000 rpm/ 5 min), and the supernatant removed. This procedure was repeated three times. Final concentration of the rabbit blood was measured by hematocrit. For this purpose, three microcapillars were filled with the washed blood and centrifuged (10 min at 5000 rpm). The average hematocrit measurement from the three microcapillars was used. Then, the rabbit blood was taken to a concentration of 1% with PBS.

In a plate of 96 wells with rounded bottoms (Corning #3795) samples were serially diluted (1:2) with PBS from column 1 to 11 resulting in a dilution range from 1 to 1/1025. 25 microL of the 1% rabbit blood solution was added to each well. The 12th column functioned as a negative control. Plates were sealed and gently mixed for 30 sec and then incubated (1602H Hova-Bater incubator) for 90 min at 31ºC for samples from the low temperature treatment, at 37ºC for samples from the control treatment, and at 39ºC for samples from the high temperature treatment. Incubation temperatures were the same as the average body temperature of the males in each temperature treatment. Immediately after incubation plates were placed at an angle of 45º for 20 min. Afterwards plates were scanned (HP deskjet F4180) and from the pictures the strongest dilution at which agglutination still took place were determined. Plates were then rested 70 min at room temperature and scanned a second time to determine maximum lysis. To determine repeatability, each plate included a pool sample and for eight samples duplicates were repeated in different plates. Repeatability between plates was high (R = 0.84 for agglutination and R = 0.90 for lysis). The dilution at which agglutination and lysis still took place was evaluated independently by two observers with high reliability intra (mean = 96% for agglutination and 99% for lysis) and inter observer (90% for agglutination and 98% for lysis). For statistical analyses the average agglutination and lysis values of the two observers was used.

Statistical analyses

Data were analyzed as repeated measures in generalized linear mixed models. Afterwards it was tested whether the distribution of the errors was parametric using the shapiro test. Separate models were performed for the different colour traits (blue chroma, lightness of the collar, area of the collar, and number of white spots on the dorsum). Initial models included treatment, body temperature, SVL, number of ectoparasites, and either agglutination or lysis. To determine whether there was a trade-off between colour and immune response, we tested for an interaction between treatment and immune response. Also, we evaluated the affect of parasites by testing the interactions of parasites x treatment, parasites x immune response and parasites x treatment x immune response. Final models were determined by stepwise backwards selection until only significant variables were included in the model (West et al. 2007).

Analyses were performed in SAS version 9.0. Mean ± standard deviations are given throughout the results.

Results

Environmental temperature and male body temperature

Environmental temperature at which males were exposed affected their body temperature, particularly for males in the low temperature treatment. Male body temperature was significantly lower in the low temperature treatment (29.5 ± 4.5ºC), compared to the control (36.6 ± 1.6ºC; Paired *t*-test = -7.59, $P < 0.01$), and high temperature treatments (37.0 ± 1.6ºC; Paired *t*-test = -8.73, $P < 0.01$, $n = 18$). Body temperature of males from the control and high temperature treatments did not differ (paired *t*-test = 0.97, $P = 0.35$), possibly because at the time measurements were taken, temperatures were high and many males in the high temperature treatment had taken refuge. Indeed, males that had not yet entered refuge had higher body temperature in the high temperature treatment (38.6 ± 0.6ºC) than in the control treatment (36.6 ± 1.6ºC; paired *t*-test = -3.98, $P = 0.001$, $n = 5$).

Effects of temperature on male immune response

Individual male's immune response varied with temperature. Both agglutination and lysis were lower at low temperature (agglutination = 3.87 ± 0.74, lysis = 3.28 ± 0.57) and high temperature treatments (agglutination = 3.66 ± 0.51, lysis = 3.13 ± 0.53) than at control treatment (agglutination= 4.95 ± 0.97, $F_{2,34} = 17.47$, $P < 0.01$; lysis = 4.53 ± 0.70, $F_{2,34} = 38.03$, $P < 0.01$; Fig 1a, b). There was no difference between agglutination and lysis measured at low and high environmental temperature (agglutination: Paired *t*-test = 1.43, $P = 0.17$; lysis: Paired t-test = 1.02, $P = 0.32$). Agglutination and lysis were highly correlated ($R = 0.86$, $P < 0.01$).

Interestingly, males that had higher agglutination or lysis values during the control treatment had a greater reduction of immune response at both low and high temperatures (control to low temperature: agglutination: $R = -0.83$, lysis: $R = -0.78$; control to high temperature: agglutination: $R = -0.70$, lysis: $R = -0.67$; $P < 0.002$).

Effects of temperature on male colour traits

Temperature influenced the male's blue dorsal colour. Blue chroma was significantly lower at low temperatures (blue chroma = 0.25 ± 0.02; $F_{2,34} = 14.41$, $P < 0.01$), but there was no difference between the blue chroma of the males in the control (0.28 ± 0.02) and high temperatures (0.27 ± 0.03; Paired *t*-test= 0.91, P = 0.37; Fig 1c). Interestingly, males that were bluer in the control treatment reduced more their blue chroma in the low temperature treatment (R = -0.61, P = 0.007), and, a similar but not significant trend in the high temperature treatment was found (R = -0.43, P = 0.07). The size of the collar and the lightness of the black stripes did not differ between treatments (black area, $F_{2,30} = 0.28$, P = 0.76; white area, $F_{2,30} = 1.87$, P = 0.19; lightness of the black stripes $F_{2,32} = 1.01$, P = 0.37). Temperature treatment also did not affect the total area of white spots ($F_{2,30} = 1.95$, P = 0.16).

Trade-off between immune response and colour mediated by temperature

Agglutination had a temperature dependent effect on blue chroma (interaction: agglutination x temperature treatment, $P = 0.01$; table 1). Also, the size of the male affected the relation between agglutination and blue chroma. Small males with higher agglutination values had lower blue chroma in control and high temperature treatment, but there was no relation in the low temperature treatment (fig 2). In big males however we found no relationship between agglutination and blue chroma in the control and high temperature treatments, but they displayed a strong positive relation in the low temperature treatment (fig 2).

Males that in the low temperature treatment maintained higher levels of agglutination (compared to control) also maintained higher blue chroma (fig 3). However, in the high temperature treatment, males that showed a greater decline in agglutination slightly increased the blue chroma, whereas males that did not change agglutination value slightly reduced blue chroma (fig 3). The lightness and size of the collar and the number of white spots on the dorsum did not appear to have such a trade-offs (all $P > 0.42$), nor did we find a trade-off between lysis and colour (interaction lysis x temperature treatment, P > 0.11).

Discussion

Environmental temperatures strongly affect body temperature and therefore the performance levels of ectotherms (Angilletta 2009). A temperature dependent skin colour could be a dynamic signal of physiological and behavioural performance level. At extreme temperatures displaying a good temperature-dependent skin colour may be costly, as animals have to maintain body temperatures that diverge from environmental temperatures. This would require more sunbathing at low temperatures, which is time consuming and makes the animals more vulnerable to predation (Huey 1982). On the other hand, when their environment reaches above optimum temperatures, animals may have to search refuge, which reduces the time they can forage or interact with conspecifics. At these stressful circumstances, trade-offs to cope with these extra costs may occur. We found that in the Dickerson collared lizard the intensity of the blue body colour was diminished and immune response was weaker at extreme environmental temperatures. Furthermore, we found that there was a trade-off between blue chroma and immune response.

In ectotherms, only recently it has been found that structural colours, such as blue, may vary with body temperature (Hettyey et al. 2009, Bajer et al. 2012, Langkilde and Boronow 2012). Here, we experimentally demonstrate that blue chroma of male Dickerson's collared lizards varies with environmental temperature. Temperature affects the space between platelets in the iridophores, which determines the wavelength that is reflected (Morrison et al. 1996). This causes a relation between body temperature and colour, in which at lower temperatures higher wavelengths are reflected and animals are greener. This temperature-dependent change in blue colour could be, when related with performance level, a dynamic signal.

Performance level of ectotherms depends on body temperature, with an optimum that should be near the preferred or normal operating body temperature (Angilletta et al. 2002, but see Martin and Huey 2008), and lower performance at higher or lower temperatures. In our study, males at the control treatment had the highest immune response, and displayed a body temperature of 37ºC, which is similar to body temperatures found in the field (Plasman et al. 2007, 2015).

Furthermore, the strength of the immune response followed a quadratic shape with a lower response at low and high environmental temperatures than at control temperatures. Similar variations of immune response at different temperatures have been found in other reptiles (Mondal and Rai 2001, Merchant et al. 2003, Dittmar et al. 2014, but see Butler et al. 2013).

As both the immune response and sexual traits are probably costly (Lochmiller and Deerenberg 2000), animals will face a compromise when maximizing the expression of both traits (reviewed in Fedorka 2014). We found that both in the control and high temperature treatment, small males with stronger immune response had a lower blue chroma. Bigger males however did not show such a trade-off. Smaller, younger males may have low chances of reproduction (Baird and Timanus 1996) and rather invest in survival (Velando et al. 2006, Fedorka 2014). At low temperatures no relation was found in small males, however, big males with stronger immune response had higher blue chroma. Trade-offs are often only appreciated under extreme circumstances (French et al. 2007, Lazzaro and Little 2009), and low temperatures may thus reveal “high quality” males, which can afford the costs associated with both immune response and coloration.

Interestingly we found that animals that displayed a higher blue chroma and stronger immune response in the control treatment decreased more these variables when moved to extreme temperatures than did animals with lower blue chroma and weaker immune response in the control treatment. Possibly enzyme kinetics at extreme temperatures limits the strength of the reaction of the natural antibodies and variance is higher in control than in high or low temperature treatments. However, the males with highest immune responses in the control treatment did not present an immune response in the upper range of the measurements in extreme temperature treatments, nor blue chroma. Thus it appears that immune condition and colour in normal conditions do not indicate the performance at extreme temperatures. Hence, it will be important to evaluate these dynamic signals in different environmental conditions.

Due to climate change average temperature is rising (IPCC 2012). Moreover there will be more fluctuations in temperature and extreme temperatures will be more common (Coumou and Rahmstof 2012; IPCC 2012). Extreme temperatures are likely to affect animals more than an increase in average temperature (e.g. Vasseur et al. 2014). We also found that even though control and high temperature treatment did not differ significantly in average temperature, immune response was lower in the high temperature treatment, probably due to the higher maximum environmental temperature or the difference in incubation temperature, which was based on body temperature. Our subject species is a diurnal desert dwelling species, active only in spring and summer (McGuire 1996) and normal operating body temperature is lower than environmental temperature. Further increases in environmental temperatures may put more strain on these animals to maintain their optimum body temperature (Clusella-Trullas et al. 2011). A slight increase in environmental temperature can lead to increases in body temperatures and can strongly reduce their performance (Angilletta 2009). We found that male Dickerson's collared lizard reduced their immune response at higher temperatures. Hence, heat waves in an already hot environment, could strongly affect the survival of this species.

In the present study we found that a sexual colour trait varies with temperature. Also, an immune response based on natural killer cells and the alternative pathway is affected by temperature. Both traits displayed higher levels at control, compared to low and high temperature treatments. We conclude that the blue dorsal colour of male Dickerson's collared lizards is a dynamic signal of immune response. Furthermore, we found that small males displayed a trade-off between the sexual colour trait and immune response. As both traits are probably expensive (Lochmiller and Deerenberg 2000), young males that have lower chances of reproduction in collared lizards (Baird and Timanus 1996) may lower the costs of displaying such a signal and invest more in their survival to a more profitable reproductive season (Fedorka 2014).

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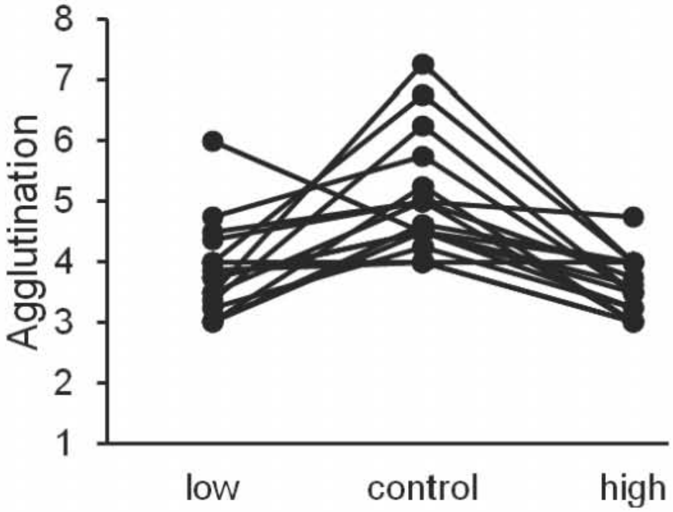
Tables

Table 1. The effects of temperature treatment, condition (snout-vent length (SVL), body condition index (BCI), number of ectoparasites) and immune response on male blue chroma. Effects were analyzed with General Linear Mixed Models with normal error distribution. Degrees of freedom (df), F-values and P-values are given for all evaluated main effects and interactions.

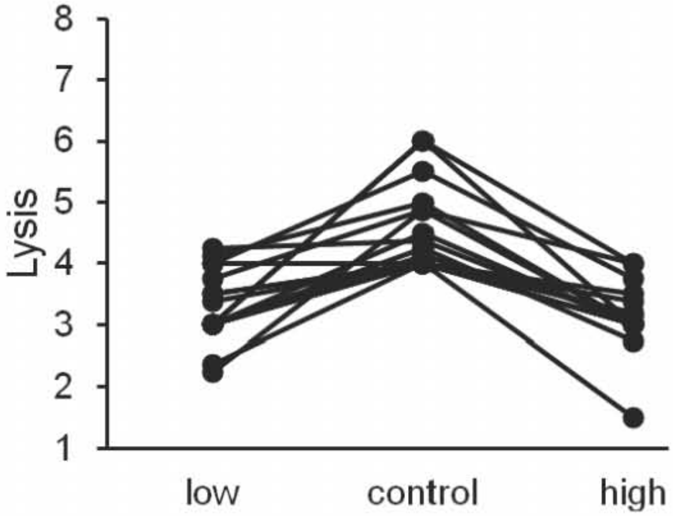
Variables included in final model	df	*F*	*P*
Treatment	2,28	4.40	0.022
Agglutination	1,28	5.71	0.024
SVL	1,16	5.90	0.027
Agglutination * Treatment	2,28	7.22	0.003
SVL x Treatment	2,28	3.86	0.033
SVL x Agglutination	1,28	5.67	0.024
Rejected variables			
BCI	1,14	0.64	0.437
Parasites	1,15	2.63	0.126
Body temperature	1,27	2.73	0.110
BCI x Treatment	2,24	1.85	0.180
BCI x Agglutination	1,21	0.18	0.680
Parasites x Treatment	2,24	0.48	0.626
Parasites x Agglutination	1,26	2.16	0.154
SVL x Treatment x Agglutination	2,26	0.95	0.400
BCI x Treatment x Agglutination	2,19	1.34	0.970
Parasites x Treatment x Agglutination	2,20	0.03	0.285

Figures

1a



1b



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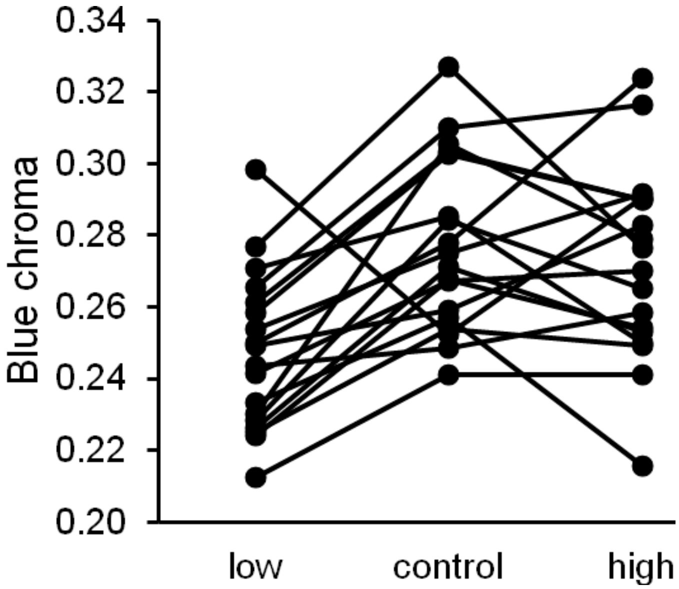

Figure 1. The effect of temperature treatments on a) agglutination, b) lysis, and c) blue chroma. Each line indicates an individual male.

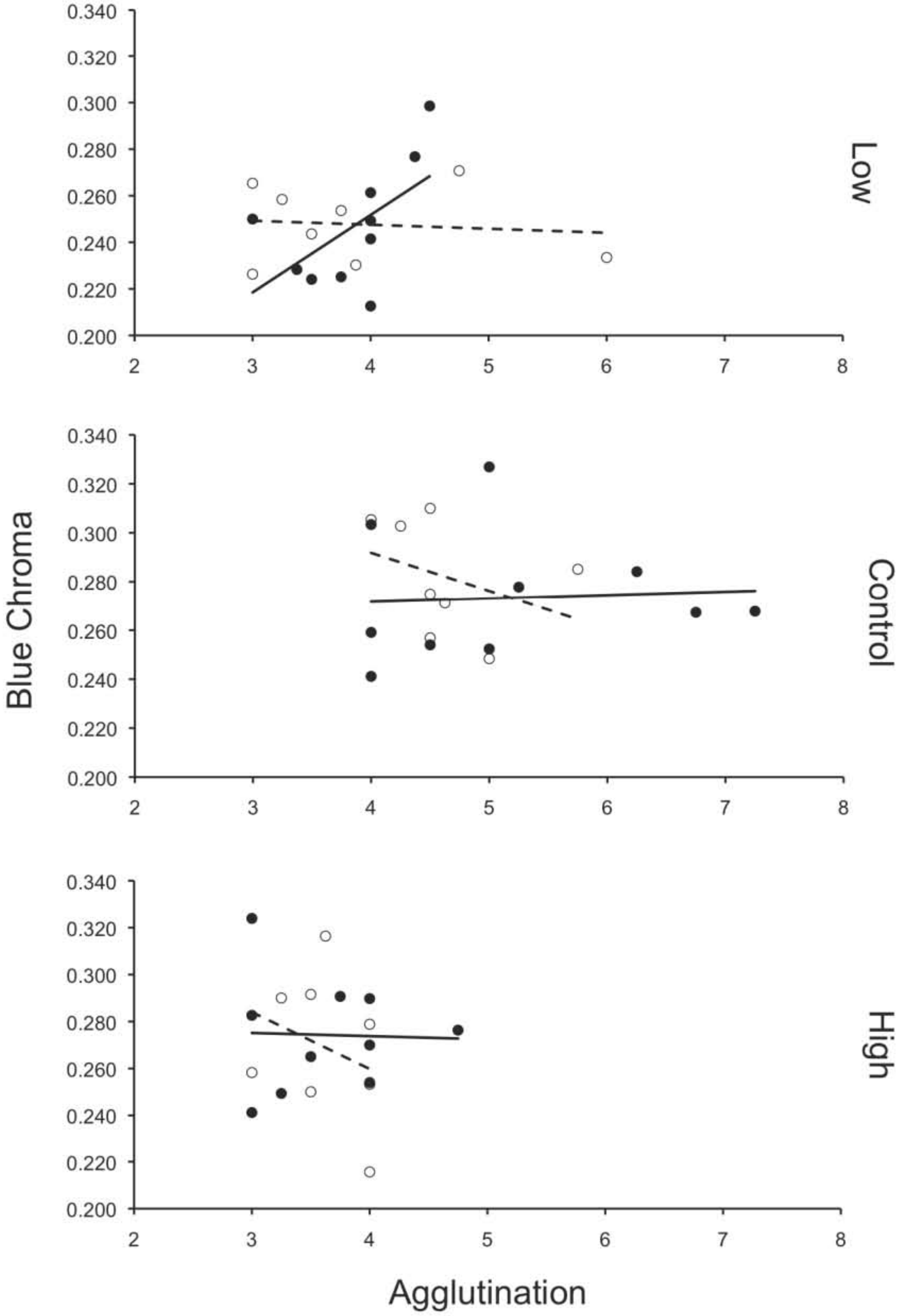
0.340
0.320
0.300
0.280
0.260
0.240
0.220
0.200
2
3
4
5
6
7
8
Low
Blue Chroma
Control
High
Agglutination

Figure 2. Size of the males had a significant influence on the relation between agglutination values and blue chroma. Relationships are displayed for the three treatments (low, control and high temperatures) for smaller (open circles and dashed line) and bigger (solid circles and continuous line) than average males.

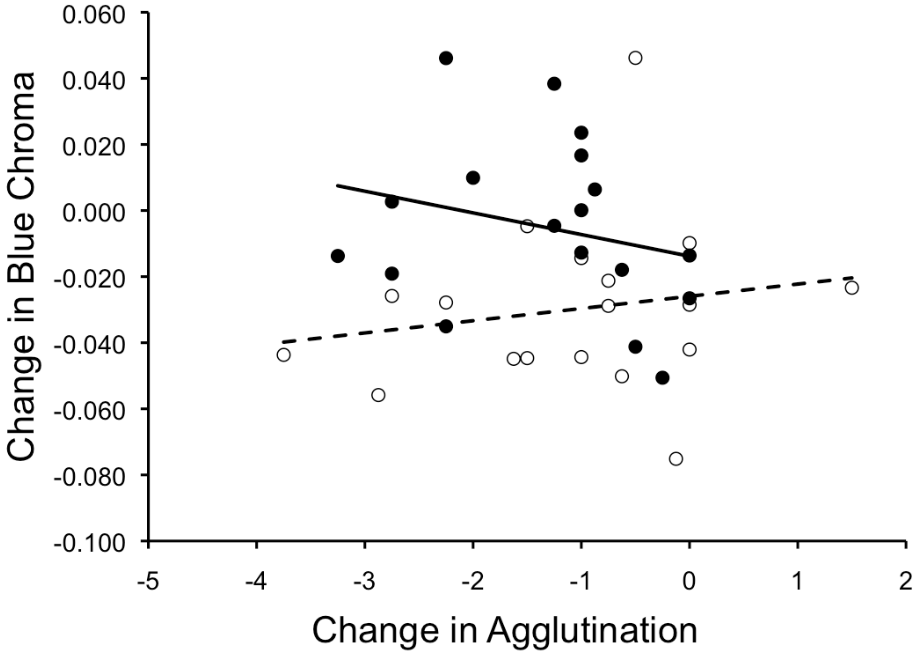

Figure 3. Relation between change in agglutination (compared to the measurement taken at the control) and the change in blue chroma (also compared to the measurement taken at the control). Open circles and dashed line indicates the relationship of change in agglutination and blue chroma at low temperatures and solid circles and continuous line indicates the relationship of change in agglutination and blue chroma at high temperatures.

Capítulo 6.

Discusión y conclusiones generales

Capítulo 6: Discusión y conclusiones generales

Entre las lagartijas hay especies con coloraciones muy llamativas, entre ellas destacan algunas en que todo el cuerpo es muy colorido. Este es el caso de la lagartija de collar de Dickerson *Crotaphytus dickersonae*. Los machos desplieguen un color azul cobalto sobre todo el cuerpo, un collar de dos bandas negras dividido por una banda blanca, y puntos blancos en el dorso (McGuire 1996). Dado que el fenotipo de color esta formado por un conjunto de características que podrían simultáneamente estar informando diferentes componentes de la condición y calidad del individuo que despliega, se podría esperar que la selección favorezca en esta especie una comunicación vía señales múltiples (Grether et al. 2004). En este estudio encontré que la coloración de los machos se relaciona con su desempeño y respuesta inmune. Machos de un color azul más intenso y un collar más grande y obscuro tuvieron mejor desempeño y respuesta inmune. Co-específicos ajustan su comportamiento por la coloración del individuo que encuentren. Las hembras se acercan más a machos con collares más pequeños y menos obscuros. La agresividad de los machos aumenta hacia oponentes más azules. Además encontré que la intensidad el color azul es dinámica. Ocasionalmente los machos se obscurecieron después de perder una pelea. Además la intensidad del azul es más baja a temperaturas bajas y altas. Asimismo, encontré que la respuesta inmune es menor a temperaturas extremas. En machos pequeños hubo un compromiso entre respuesta inmune e intensidad del color sexual. Los resultados indican que en la lagartija de collar de Dickerson la coloración llamativa es una señal honesta de calidad la cual es usada en las interacciones con co-específicos.

Coloración de la lagartija de collar de Dickerson como señal honesta de calidad y condición del individuo

En los machos de lagartija de collar de Dickerson los múltiples parches de color señalan desempeño y condición inmune. En mi estudio encontré que los machos con un azul más intenso, un collar más grande y más obscuro, y más puntos blancos, tuvieron más fuerza de mordida y mejor desempeño locomotor. Estas características se relacionan con las habilidades competitivas de los machos ya que las peleas de lagartijas involucran persecuciones rápidas y mordidas (Robson y Miles 2000, Perry et al. 2004, Huyghe et al. 2005, Husak y Fox 2006, Husak et al. 2006a,b). En otras especies de lagartijas de collar, los machos con más fuerza de mordida y que corren más rápido son más dominantes y tienen un éxito reproductivo más alto (Husak et al. 2006a,b, 2008, 2009). Las señales que informan sobre la fuerza de mordida y velocidad del individuo podrían disminuir los costos de pelear, ya que los machos pueden decidir pelear sólo cuando sus probabilidades de ganar son altas (Maynard-Smith y Price 1973, Rohwer 1982, Olsson 1994). Los colores conspicuos que señalan características tales como la fuerza y la velocidad podrían permitir la evaluación de la capacidad de retención de los recursos del individuo desde una mayor distancia.

También encontré que machos con un collar más obscuro tenían menos parásitos y una mejor respuesta inmune, lo que sugiere que el collar indica estatus inmune. Una buena respuesta inmune probablemente está relacionada con buenos genes resistentes a las enfermedades (Hamilton y Zuk 1982) y un collar obscuro puede señalar alta calidad. Además, una buena respuesta inmune puede incrementar la adecuación cuando, por interacciones entre machos, la probabilidad de heridas es alta (Zuk y Johnson 1998). Curiosamente encontré que los machos que tenían una carga de ectoparásitos mayor tuvieron una mejor respuesta inmune. Esto podría ser el resultado de preparación del sistema inmune, cuando los parásitos por sus ataques al huésped hacen más sensible y eficiente los mecanismos del sistema inmune en ataques posteriores (Huyghe et al. 2010, Schmid-Hempel 2011). Por otro lado encontré que los machos más grandes montaron una respuesta inmune más baja que los machos de tallas más pequeñas; lo anterior sugiere inmuno-senescencia, ya que lagartijas crecen todo su vida y los machos más grandes probablemente son más viejos (Sexton et al. 1992). De acuerdo con las predicciones, encontré que la temperatura corporal juega un papel importante en el desempeño de algunas características fisiológicas de la lagartija de collar. Encontré que los machos con temperaturas corporales más bajas tenían una respuesta inmune más baja, lo que indica que la respuesta inmune depende de la temperatura (Zimmerman et al. 2010). La respuesta inmune no únicamente depende de los buenos genes, sino también de la edad, el estatus inmune anterior y la temperatura corporal.

Contrario a lo esperado, no encontré evidencia de que la testosterona influya en el color, el desempeño o la respuesta inmune, de tal manera que mis datos no apoyan la hipótesis del hándicap de inmunocompetencia (Folstad y Karter 1992). En mi estudio todos los machos tenían coloración adulta completamente desarrollada. Puede ser que la testosterona sólo afecta el color durante su desarrollo (Kimball y Erpino 1971, Hews et al. 1994, Hews y Moore 1996) y que en la lagartija de collar la coloración indica nivel de testosterona durante el desarrollo. Además puede variar el nivel de testosterona con el estrés, la condición inmune o la edad (Moore et al. 1991, Folstad y Karter 1992). Encontré que el nivel de testosterona estaba relacionado con la temperatura corporal y también la respuesta inmune fue afectada por la temperatura y el tamaño corporal. Es importante conocer la variación entre individuos en edad y condición para poder entender los posibles compromisos entre colores que dependen de la testosterona y el desempeño fisiológico (Alonso-Alvarez et al. 2009, Ruiz et al. 2010), pero por el momento no tenemos evidencia que la testosterona afecta el color o la respuesta inmune.

Una señal honesta se relaciona con condición y/o desempeño del individuo y puede ser utilizado por co-específicos para evaluar estos características del individuo (Guilford y Stamp Dawkins 1991). Encontré que el color azul y el collar negro indican capacidad de retención de los recursos. Además el collar indica respuesta inmune y puede indicar los buenos genes. De este manera la coloración de la lagartija de collar existe de múltiples señales que pueden ser usados en la competencia entre machos y elección femenina.

¿Influye el color en la elección femenina?

En teoría, las hembras pueden aumentar su adecuación al escoger un macho de buena calidad, ya que sus hijos heredan los buenos genes (Zahavi 1975; Hamilton y Zuk 1982; Kodric-Brown y Brown 1984; Grafen 1990). La coloración de los machos de la lagartija de collar de Dickerson señala capacidad de retención de los recursos y estatus inmune, información que las hembras podrían usar en su elección de pareja. En lagartijas, las hembras pueden basar su elección de pareja en señales múltiples (Lancaster et al. 2009, Swierk et al. 2012), que pueden incluir señales de diferentes parches o diferentes aspectos de los parches de color (Candolin 2003, Grether et al. 2004), o también pueden incluir otros aspectos morfológicos o conductuales.

Encontré que varios aspectos de la coloración del macho afectaron la conducta de preferencia por parte de las hembras. Aunque las hembras desplegaron más hacia machos con collares negros obscuros, se acercaron más y quedaron más tiempo cerca de machos con un collar más luminoso y pequeño. Despliegues de los machos parecen aumentar la eficiencia de evaluación de señales, ya que en machos con collares aumentados estos despliegues disminuyeron el acercamiento de las hembras. Sin embargo estás características relacionaron con peor calidad ya que machos con collar más luminoso y pequeño tenían velocidad, fuerza de mordida y respuesta inmune baja (vea capítulo 1). Lo anterior podría resultar si las hembras al elegir pareja tratan de reducir los costos asociados a la cópula (Qvarnström y Forsgren 1998, Lessells 2005), ya que en lagartijas los machos pueden morder a las hembras durante la cópula (Baird et al. 2003, Huyghe et al. 2012). Alternativamente, las hembras podrían estar evaluando otras cualidades del macho que para nosotros son desconocidos, como por ejemplo la edad (Richard et al. 2005). Por otro lado, cuando el color azul fue medida inmediatamente antes del ensayo, las hembras se acercaron más a machos más azules. Machos de color azul más fuerte y collar más pequeño y luminosa tenían temperaturas corporales más altas, que puede indicar un mejor desempeño físico y fisiológico (Angilletta et al. 2002).

En la vida libre, el territorio del macho probablemente afecta la elección femenina (Tokarz 1995) y en las lagartijas de collar calidades relacionados a capacidad de la retención de los recursos (velocidad y fuerza de mordida) se relacionan con el éxito reproductivo de los machos (Husak et al. 2006a,b, 2008, 2009). Esto puede disminuir el efecto de elección femenina y la competencia entre machos podría ser un mecanismo más importante para la adecuación de los machos.

¿Influye el color en la competencia ente machos?

Los machos con una coloración azul más intensa y collares más obscuros y grandes tuvieron un mejor desempeño en fuerza de mordida y velocidad, características relacionados con capacidad de retención de los recursos. Por lo tanto, esperaba que en encuentros con otros machos, los machos usaran la coloración del macho oponente para determinar la intensidad de agresión. Encontré que ni la coloración azul, ni el collar negro determinó el ganador de un encuentro entre dos machos. Sin embargo, los machos que tenían un color azul más intenso fueron más agresivos que machos menos azules y los machos dominantes fueron más agresivos hacia machos de color azul más intenso. Por otro lado, los subordinados que tenían un collar blanco más grande desplegaron más conductas sumisas. Al manipular el tamaño del collar, los subordinados desplegaron más conductas sumisas hacia sus oponentes cuando estos tenían el área del collar disminuido. Sin embargo, un collar más grande se relacionaba con más fuerza de mordida y velocidad. De este manera podría ser que el collar señale algún otro atributo que no fue evaluado. Por ejemplo, la experiencia es muy importante para las oportunidades de ganar y los machos más viejos probablemente tengan más experiencia y frecuentemente sean dominantes (Smith 1985, Aragón et al. 2004, Wikelski et al. 2005). Así que si exista una señal que indica edad puede ser más importante que la fuerza de mordida o velocidad.

Interesantemente observé que en algunos casos el macho sumiso disminuyó drásticamente el croma azul, poniéndose de un color café. Un cambio en color como efecto de perdedor, también ocurre en la lagartija *Anolis carolinensis* que cambia de verde a café cuando pierden una pelea (Greenberg y Crews 1990). El cambio en croma azul a corto plazo indica que este color puede ser una señal dinámica en la competencia entre machos, señalando dominancia cuando es azul intenso y estatus subordinado cuando se pone de color café.

Color como señal dinámica

En la lagartija de collar de Dickerson el color azul no es un color estática, sino que puede cambiar en función de las condiciones sociales y ambientales. Encontré que machos con temperatura corporal más alta, tenían el croma azul más alto y un collar más pequeño y luminoso, que indica que estos parches de colores posiblemente varían con la temperatura. Además, la temperatura corporal afectó las variables fisiológicas. Machos con temperaturas corporales más altos eran más grandes, tuvieron una respuesta inmune más fuerte y niveles de testosterona más altos. Ya que en ectotermos la temperatura afecta el desempeño y respuesta inmune (Angilletta 2009), un color que depende de la temperatura podría funcionar como una señal dinámica del desempeño fisiológico y físico. En otras lagartijas también se ha encontrado una relación de temperatura con colores obscuros (revisado en Clusella-Trullas et al. 2007) y estructurales (Morrison et al. 1996, Bajer et al. 2012, Langkilde y Boronow 2012), sin embargo falta investigar posibles funciones en la comunicación intraespecífica de estos colores dependientes de la temperatura.

Tanto las características sexuales como la respuesta inmune probablemente son costosos de mantener (Lochmiller y Deerenberg 2000) y los animales tendrán que hacer un compromiso y dividir sus recursos entre estos dos características (Fedorka 2014). Evalué si exista el compromiso entre respuesta inmune y la coloración del macho mediado por la temperatura. Encontré que en la lagartija de collar, a temperaturas normales y altas machos pequeños con respuesta inmune más fuerte tenían peor coloración azul. Machos pequeños pueden tener una probabilidad baja de reproducción (Baird y Timanus 1996) y preferentemente invertir en la supervivencia (Velando et al. 2006, Fedorka 2014). En los machos grandes no hubo tal compromiso. Además a temperaturas bajas los machos grandes que tenían una respuesta inmune más alta, tenían también un croma azul más alto. Los resultados sugieren que los machos grandes son de mejor calidad y pueden pagar los costos de las dos características (color y respuesta inmune), mientras que los machos más chicos no pueden pagar los costos de ambos características y tienen que hacer un compromiso entre coloración sexual y respuesta inmune.

Conclusiones generales

La coloración de los machos de la lagartija de collar de Dickerson, *Crotaphytus dickersonae*, parece ser una señal honesta de la capacidad de retención de recursos y estatus inmune, y podría jugar un papel importante en la comunicación intraespecífica, en particular en la competencia entre machos. La coloración en la lagartija de collar esta conformada por varios parches de color, es decir un sistema multiseñal, además incluye una señal dinámica que puede cambiar en periodos de tiempo cortos en función del contexto social y ambiental. La temperatura corporal en esta especie no sólo afecta variables fisiológicas, como la respuesta inmune y los niveles de testosterona, sino también el color que despliegan los machos. Concluimos que la coloración conspicua de los machos de la lagartija del collar son señales honestas de su desempeño y condición fisiológica, y afecta el comportamiento de sus co-específicos hacía ellos. Muchas lagartijas desplieguen colores llamativos, los cuales pueden formar parte de un complejo sistema de señales múltiples que indican la calidad del individuo. Además, estas señales pueden ser más dinámicas que antes pensado. Señales de color pueden variar con la condición del animal, pero también con su temperatura corporal. Por lo tanto la temperatura corporal no sólo afecta el desempeño fisiológico y físico de los ectotermos, sino también, cuando afecta coloración, la comunicación intraespecífica.

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