EVOLUCIÓN DE LAS SEÑALES IMPLICADAS EN LOS PROCESOS DE SELECCIÓN SEXUAL DE LAS LAGARTIJAS SERRANAS IBÉRICAS

(Género Iberolacerta)

Carlos Cabido

Tesis Doctoral

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La comunicación animal es consecuencia de un proceso de selección natural, siendo influenciada por múltiples factores de forma compleja y diferente en cada especie o población. El uso de señales sexuales, al influir, tanto directamente en los procesos de selección sexual y reproducción como indirectamente en la supervivencia de los individuos, constituye un factor determinante en los procesos evolutivos. Además, las señales sexuales, al mediar los procesos de aislamiento reproductor precópula, son determinantes, no sólo de muchos procesos de especiación, si no del propio concepto biológico de especie.

El objetivo general de esta Tesis es el de explorar las influencias que el ambiente puede tener sobre la evolución de las señales implicadas en los procesos de selección sexual de las lagartijas serranas ibéricas. Entendemos "ambiente" como el escenario ecológico sobre el que actúa la selección natural, compuesto por factores tanto abióticos como bióticos. En particular, nos interesa saber qué factores pueden promover la divergencia entre poblaciones en cuanto al uso de distintos tipos de señales, y las consecuencias evolutivas de la triple interacción entre ambiente, comportamiento y señales, siempre en un contexto evolutivo.

El ambiente físico o social (sistema de apareamiento) puede mediar la variación microgeográfica en la utilización de señales sexuales visuales o químicas entre poblaciones de lagartija carpetana (*Iberolacerta cyreni*). Además, aunque las hembras de lagartija carpetana prefieren asociarse con las señales químicas de los machos de mejor calidad (medida como una mejor respuesta inmune), ésta está correlacionada con distintos compuestos de sus señales en diferentes poblaciones aisladas geográficamente. Esto resulta en la preferencia de las hembras por machos simpátricos, lo cual podría conducir a un eventual proceso de aislamiento reproductor y especiación. Las hembras de lagartija cantábrica (*Iberolacerta monticola*) también prefieren las señales de los machos con mejor respuesta inmune. Sin embargo, al contrario que en la lagartija carpetana, en esta especie más territorial, los rasgos de las señales químicas relacionados con la dominancia también son preferidos por las hembras. Así, al determinar el ambiente social los beneficios directos y/o indirectos que los machos proveen a sus parejas pueden, por tanto, sesgarse las preferencias de las hembras por diferentes rasgos de las señales de los machos.

La depredación también puede influir, tanto en la evolución de distintos tipos de señales, como en el comportamiento asociado a su uso. Los machos de lagartija cantábrica modulan su comportamiento antidepredatorio para compensar los efectos negativos de un incremento del riesgo de depredación debido a la coloración sexual conspicua, mientras que los machos de lagartija cantábrica con mayor "atractivo químico" para las hembras muestran un mayor interés en el olor de una serpiente depredadora que utiliza estímulos químicos de las lagartijas para cazar. Todo esto sugiere que la regulación del comportamiento antidepredador en función de la propia conspicuidad puede influir en los costes asociados al uso de señales explicando la persistencia de un tipo u otro de señales en distintas especies ■

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Memoria presentada por el Licenciado en Biología Carlos Cabido Quintas
para optar al título de Doctor en Biología por la Universidad de A Coruña

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Pedro Galán Regalado
Universidade da Coruña

A mis padres y hermana
A Adega

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Por alguna extraña razón, siempre me gustaron "los bichos". Lejos de ser una mera etapa, es algo que se convirtió en una constante que ha acabado desembocando, por ejemplo, en esta Tesis doctoral. El haber podido satisfacer toda mi curiosidad por los animales desde la infancia, es algo que les debo a mis padres. Ellos soportaron, a regañadientes pero con paciencia, todo mi "ensimismamiento", todos los animales que realizaban "estancias" conmigo (pero en su casa) e, incluso, sus fugas o problemas logísticos. Su apoyo para que, primero me licenciara en Biología y, después, prolongara mi infancia indefinidamente al decidir que quería dedicarme a la investigación, ha sido esencial para poder llegar hasta aquí. En ese contexto, sé que también mi hermana, con su ciego apoyo, ha servido de mediadora en las tensiones que, algunas veces, surgían por culpa de mi carácter y peregrinas ambiciones. Otras personas me han apoyado porque han creído que podría aportar alguna cosa a algo tan complejo como es la ciencia. Sin embargo, mis padres y hermana, ajenos a este mundo, simplemente "creyeron en mí", lo cual agradeceré eternamente.

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Introducción

LA COMUNICACIÓN EN EL CONTEXTO DE LA ECOLOGÍA DEL COMPORTAMIENTO

> “*We are drowning in information, while starving for wisdom.*
>
> Edward Osborne Wilson (Consilience: The Unity of Knowledge, 1998)

La mayor parte de los animales influencian o se ven influenciados por otros animales, de su misma o de diferente especie, a través de la transferencia de información. La información puede ser enviada a individuos de la misma especie, a depredadores o, incluso, al mismo individuo, e intervenir en diferentes fenómenos sociales, de navegación y selección del hábitat o de depredación. Parte de esa transferencia de información puede considerarse comunicación. Así, los sistemas de comunicación han evolucionado porque los animales pueden y tienen que tomar decisiones basadas en el comportamiento, fisiología o morfología de otros individuos de su misma o diferente especie, a través del uso de señales (Endler 1993). Desde la perspectiva de la Ecología del Comportamiento y la Sociobiología, la comunicación es lo que une cualquier sociedad animal, teniendo un papel central en los procesos que condicionan en gran medida la evolución de las especies. Por ejemplo, la reproducción y los procesos de selección sexual son imposibles sin la comunicación y el uso de señales y los animales suelen presentar sofisticados rasgos para comunicarse la información necesaria para el apareamiento y otras actividades. Incluso en especies solitarias o poco sociales es necesario el uso de señales elaboradas para mantener esos patrones de dispersión espacial.

Tinbergen (1963) sugirió que, ante cualquier rasgo morfológico o comportamental, pueden plantearse cuatro cuestiones independientes en cuanto a mecanismo, ontogenia, filogenia o función. La última cuestión, la función, referida al valor adaptativo (también conocida como “causas últimas”), es la que protagoniza todos los capítulos de la presente Tesis Doctoral, aunque tanto el mecanismo (“causas próximas”), como la filogenia subyacen, en mayor o menor medida, la mayor parte. La evolución es un proceso, consecuencia de la selección natural, que depende de la eficacia biológica (reproducción diferencial) de los diferentes individuos de una población interconectada. En ese contexto, la comunicación animal es consecuencia de un proceso de selección natural, siendo influenciada por múltiples factores, de forma compleja y diferente en cada especie (Darwin 1872). Por ejemplo, la selección natural optimizará las señales en función de las características físicas del medio, de la detección por parte de los depredadores o de la organización social de cada especie. Además, el uso de señales, al influir, tanto directamente en los procesos de selección sexual y reproducción, como indirectamente en la supervivencia de los individuos, constituye un factor determinante en la eficacia biológica y, por tanto, en los procesos evolutivos. Finalmente, dado que las señales sexuales median los procesos de aislamiento reproductor precópula, son determinantes, no sólo en los procesos de especiación, si no en el propio concepto biológico de especie.

La comunicación incluye necesariamente la interacción de dos sistemas distintos dentro de la misma especie, un emisor y un receptor, que pueden estar sometidos a muy diferentes presiones y restricciones evolutivas (Endler 1993). La presencia de estos componentes distintos que tienen necesariamente que interaccionar, su implicación en los procesos de selección sexual y la multitud de factores de los que depende, hacen que la evolución de la comunicación intraespecífica sea un problema especialmente estimulante para la Ecología del Comportamiento. De hecho, la evolución de la forma de las señales y de las preferencias de los receptores ha recibido recientemente mucha atención por parte de los ecólogos del comportamiento, sobre todo en el contexto de la selección sexual (revisado en Bradbury y Vehrencamp 1998; Endler 1992, 1993). Sin embargo, las investigaciones sobre el uso de señales no se reparten de forma homogénea en cuanto a temática y grupos animales: La mayor parte de los trabajos se han centrado en el diseño estratégico de las señales (mecanismos que confieren honestidad; Maynard-Smith y Harper 2003), en señales visuales y en determinados grupos, dependiendo de la temática. La señalización acústica y, sobre todo, la señalización química (Wyatt 2003) han recibido, en general, menos atención. En ese sentido, llama la atención la escasez de trabajos sobre aspectos referentes al diseño estructural de las señales químicas (pero ver Alberts 1992) o de los factores que afectan al de las señales visuales de aves (pero ver Gomez y Théry 2004). De igual modo, la comunicación sexual ha sido, en general, menos explorada en reptiles, con respecto a otros grupos. No obstante, los reptiles son un grupo muy diversificado, con sofisticados y variados sistemas de selección sexual, complejas interacciones ecológicas y una fuerte dependencia del medio (Pleguezuelos 1997; Zug et al. 2001). Además, sus interacciones sociales se basan en diferentes sistemas sensoriales, incluso dentro de una misma especie (Gans y Crews 1992; Zug et al. 2001). Todo ello les convierte en un grupo muy adecuado para abordar estudios sobre evolución de señales, como ya han demostrado algunos trabajos (por ej., Whiting et al. 2003; Martín y López 2000a; Martín y López 2006; Sinervo et al. 2006), presentando algunas ventajas (capacidad de dispersión limitada; facilidad de captura, manejo y mantenimiento en cautividad, etc.) con respecto a otros grupos ■

LOS CONCEPTOS BIOLÓGICOS DE COMUNICACIÓN Y SEÑAL

Tradicionalmente se ha aceptado que la comunicación es un proceso en el cual los animales usan sus órganos sensoriales para enviar y recibir información sobre el mundo (Darwin 1872). Sin embargo, debido a la implicación de los sistemas de comunicación en gran parte del comportamiento animal, existen multitud de revisiones e, inevitablemente, cierta confusión en cuanto a la definición de qué son, tanto la comunicación como las señales, en el contexto de la Ecología del Comportamiento (ver Bradbury y Vehrencamp 1998; Maynard-Smith y Harper 2003). Esta diversidad de criterios proviene del intento de incluir todos los casos de comunicación en una sola definición. Por ejemplo, Wiley (1994) define la comunicación como cualquier alteración de un individuo por mediación de una señal. Sin embargo, Wilson (1975) matiza que supone una acción por parte de un individuo que altere el comportamiento de otro en un contexto adaptativo. Esta matización implica que para hablar de comunicación deberemos tener en cuenta el origen de la señal y cómo responde el receptor de la misma. Otros autores distinguen entre "**comunicación verdadera**", "**información robada**" ("eavesdropping") y "**coacción**", dependiendo de si la transferencia de información beneficia a ambos, emisor y receptor, sólo al primero, o sólo al segundo (Marler 1977), respectivamente. Finalmente, un tercer criterio hace referencia a la honestidad de la información transferida, que dependerá de si los intereses de emisor y receptor divergen o coinciden, generando diferentes escenarios evolutivos de favorecimiento mutuo de transferencia de la información o de "carreras de armamentos" que aseguran la honestidad de esa información (Maynard-Smith y Harper 2003). En cualquier caso, todos los autores consideran que la comunicación consiste en la provisión de información por parte de un emisor a un receptor y el subsiguiente uso de esa información por parte de éste último para decidir su respuesta (Bradbury y Vehrencamp 1998) o, más sencillamente, podemos decir que se produce comunicación, cuando se puede demostrar que el comportamiento de un animal tiene un efecto en el comportamiento de otro (Dawkins 1995).

El vehículo por el que se transfiere esa información, se denomina "**señal**". Sin embargo, también existe cierta confusión sobre la definición de señal y los diferentes términos ingleses usados (ver Cuadro 1). Maynard-Smith y Harper (2003) sintetizan que una señal es cualquier acto o estructura que altere el comportamiento de otros organismos, matizando que debe haber evolucionado debido a ese efecto y que es efectiva porque la respuesta del receptor también ha evolucionado. Esta definición excluye como señales a aquellos rasgos que, aunque sean utilizados para adquirir información sobre un individuo, no han evolucionado para tal efecto (lo que en inglés se denominan "**cues**"). Las "señales" serían aquellas implicadas en los actos de "comunicación verdadera", mientras que las "cues", que podríamos traducir por "indicaciones", serían aquellas utilizadas en los procesos de "interceptación" (Seeley 1998), pudiendo ser rasgos fenotípicos (morfológicos, acústicos, olfativos, comportamentales, etc.) o recursos defendidos o producidos por el emisor, como el nido o el territorio. Estos rasgos pueden haber surgido en otros contextos ajenos a la comunicación y ser mantenidos debido a otras presiones selectivas diferentes a la selección de los procesos de comunicación. Por ejemplo, el tamaño corporal o la velocidad de carrera pueden estar bajo la presión evolutiva de la supervivencia, pero, simultáneamente, ser utilizados como indicadores de la eficacia biológica. Sin embargo, a menudo estos rasgos son modificados para la comunicación o acaban teniendo sólo función comunicativa, tras un proceso evolutivo de ritualización, pasando a ser "señales" ■

Cuadro 1

En función de las distintas consideraciones del concepto biológico de comunicación en un contexto adaptativo, podemos definir diferentes tipos de señales y términos asociados:

Comunicación: Rasgo o acción por parte de un individuo que altera el comportamiento de otro en un contexto adaptativo (Wilson 1975).

Coacción: Rasgo o acción que altera el comportamiento de un individuo, habiendo evolucionado por sus efectos, pero siendo efectiva por razones distintas de las que han provocado su evolución (Scott-Philips 2008).

Señal (verdadera): Un acto o estructura que altera el comportamiento de otro organismo (respuesta) y que ha evolucionado para ese propósito, siendo efectiva porque la respuesta del receptor también ha evolucionado (Bradbury y Vehrencamp 1998; Maynard-Smith y Harper 2003; Scott-Philips 2008).

Indicación o rastro ("cue"): Una característica del mundo, animado o inanimado, que puede ser usada por un animal como una guía de una futura acción (Hasson 1994).

Índice o señal basada en el rendimiento: Una señal cuya intensidad está causalmente relacionada con la cualidad señalada que no puede ser falseada (Maynard-Smith y Harper 2003).

Handicap, señal zahaviana o estratégica: En contraposición a la anterior, se trata de una señal cuya fiabilidad es asegurada debido a que su coste es mayor que los requeridos solamente por cuestiones de eficacia. Son señales costosas de producir o con consecuencias costosas (Zahavi 1975).

Señal de coste mínimo: Se trata de un tipo de señal cuya fiabilidad no depende de su coste (no es un handicap) y que puede ser producida por la mayor parte de una población (no es un índice).

Coste: Pérdida de recursos como resultado de un proceso de señalización. Incluye:

Coste de eficacia: Coste necesario para asegurar que la información se transmite eficazmente (Guilford y Dawkins 1995).

Coste estratégico: Coste necesario, a través del principio del handicap (Zahavi 1975), para asegurar la honestidad de la señal (Grafen 1990a,b).

Amplificador: Rasgo o característica que no transmite información, pero que facilita la percepción o examen de una señal (Hasson 1991; 1994).

LA COMUNICACIÓN SEXUAL

“A great deal of social behaviour, including sexual selection and social dominance, can plausibly be viewed as signalling.

Alan Grafen, 1990

Darwin (1872) propuso el término de **selección sexual** para explicar la evolución de las características que proporcionan un beneficio a su portador sólo en relación con la reproducción y no pueden ser explicadas como productos de la selección natural. Estas características proporcionan dicho beneficio en uno de los siguientes contextos: aumentando la habilidad de su portador para tener éxito en la competencia directa por las posibles parejas, o aumentando su atractivo para los miembros del sexo opuesto. Møller (1994) sintetiza que la selección sexual no es más que comunicación entre machos y hembras, denominándose **señales sexuales** las señales utilizadas para facilitar o modular esas interacciones sexuales entre individuos de la misma especie.

Además de las funciones de promover el emparejamiento y evitar la endogamia, las señales sexuales median el comportamiento de cortejo y elección de pareja o la competencia entre individuos de un mismo sexo para acaparar las parejas (Andersson 1994). Existe una amplia evidencia de que las características de las señales sexuales entre los emisores y la receptividad a éstas entre los receptores varían dentro de una población (Andersson 1994; Bradbury y Vehrencamp 1998). La selección sexual se genera si esa variabilidad se traduce en diferencias en el éxito de emparejamiento entre los emisores. Dentro de la selección sexual podemos distinguir dos procesos más o menos independientes: la competencia intrasexual y la elección de pareja. La función de las señales sexuales estará, en principio, determinada por estos dos procesos, pero el contenido de la señal puede variar también dentro de cada uno de ellos, en función de las características sociales intrínsecas a cada especie. Así, la competencia intrasexual será mediada por señales agonísticas, señales de estatus, etc., es decir, señales que transmiten información a otros machos sobre su capacidad competitiva o sus intenciones (Andersson 1994). Las señales que median los procesos de elección de pareja transmiten, dependiendo del mecanismo que las origina y mantiene, información al sexo opuesto sobre distintas características beneficiosas del fenotipo (incluyendo características del territorio, recursos) o del genotipo. Sin embargo, existen distintos mecanismos para explicar los procesos de elección de pareja (no necesariamente exclusivos entre sí) que determinarán el tipo de información concreto que trasmiten las señales (señales indicadoras) o su ausencia (señales fisherianas).

Debido a la ya comentada correspondencia entre selección sexual y comunicación sexual, la propia naturaleza y evolución de las señales sexuales dependerá de los mecanismos que explican los distintos procesos de elección de pareja y los beneficios que obtienen emisor y receptor (ver también *Ecología y Evolución de las Señales Sexuales: diseño funcional*). En este contexto, podríamos clasificar las señales de elección de pareja como "**indicadoras",** si el receptor adquiere algún tipo de información "útil" sobre el emisor; o "**no indicadoras"**, si sólo se facilita la localización o presencia del emisor (ver Imagen 1).

Señales indicadoras: beneficios directos e indirectos

Las señales indicadoras transmiten una información sobre el emisor que el receptor utilizará para tomar una decisión con respecto a aquel. Normalmente el tipo de información dependerá de las características concretas de cada especie y qué le resulte "beneficioso" a la hora de seleccionar una pareja. Se habla de **beneficios directos** cuando la señal informa sobre determinados recursos que el emisor proporciona a la hembra y su descendencia (alimento, territorio, etc.); mientras que los **beneficios indirectos**, también conocidos por "buenos genes" ("good-genes"), se refieren a la "calidad" fenotípica o genética que heredará la descendencia del emisor (Andersson 1994).

Las señales varían mucho en cuanto a contenido de información y la "honestidad" o "fiabilidad" de ésta (Dawkins y Guilford 1991). Aunque el "engaño" ocasional no tiene por qué alterar el sistema de señalización, la información transmitida debe ser honesta (Johnstone y Grafen 1993; Johnstone 1997). La estabilidad de los sistemas de comunicación depende pues de los mecanismos que aseguren esa honestidad. La mayor contribución al respecto fue la propuesta del "Principio del Handicap", concebido originalmente por Zahavi (1975) y formulado posteriormente por Grafen (1990). Existen dos interpretaciones teóricas de dicho principio. El "**handicap estratégico**" o "dependiente de la condición" supone que para el mantenimiento de la honestidad debe haber un coste asociado, de forma que sólo los individuos de alta calidad (o muy motivados) puedan asumirlo (Enquist 1985). Por lo tanto, los beneficios de emitir la señal sólo superarán a los costes en los individuos superiores, de forma que la selección natural favorecerá la expresión "dependiente de la condición" de las señales (Johnstone 1985; Maynard-Smith y Harper 2003). Otra interpretación dice que las señales pueden no ser costosas porque son imposibles de "simular" (índices), de forma que las señales costosas habrán evolucionado para realzar la expresión del rasgo ("**handicap revelador**"; Johnstone 1985; Iwasa et al. 1991). Los costes de la señal pueden referirse a los costes de producción o de mantenimiento (por ej., Veiga y Puerta 1996) y deberse a múltiples factores. Por ejemplo, la mayor conspicuidad ante los depredadores (Zuk y Kolluru 1998), la asignación de recursos al sistema inmune (Salvador et al. 1996; Olsson et al. 2000) o los costes sociales (Rohwer 1982) convierten a muchas señales en costosas para los individuos de calidad más baja o en circunstancias adversas.

Señales no indicadoras: selección desbocada de Fisher y sesgos o explotación sensorial

Aunque las señales no indicadoras o "fisherianas" no transmiten ninguna información respecto a las características del emisor, en realidad, podría decirse que informan sobre la presencia o localización de éste, puesto que su función es atraer a las hembras más que los competidores. Fisher (1930) propuso que la ventaja evolutiva que el emisor obtiene de responder a estas señales consiste en que su descendencia también heredará esa misma capacidad de atracción ("**hipótesis del hijo sexy**" o "sexy sons") o la tendencia a sentirse atraída. La asociación entre ambos rasgos comportamentales establecerá un proceso de retroalimentación positiva ("**selección desbocada**" o "runaway") que hará el sistema de selección sexual (y comunicación) estable (Lande 1981; Kirkpatrick 1982).

Imagen 1. Distinguir entre señales indicadoras y fisherianas puede ser, a veces, difícil. Por ejemplo, en el caso de los sapos parteros (*Alytes obstetricans*), las señales acústicas se considera que indican el tamaño del macho y que las hembras seleccionan los de mayor tamaño, que pueden acarrear más huevos (Bosch y Márquez 1996). Sin embargo, también pudiera ser que las hembras simplemente acudieran a las señales más audibles, independientemente del valor adaptativo del tamaño del macho (señal fisheriana).

Sin embargo, para que el sistema comience, es necesario algún tipo de sesgo o tendencia innata por parte del receptor. Se han propuesto tres modelos muy similares para explicar el origen de estos sesgos que difieren sobre todo en dónde ponen el énfasis (Endler y Basolo 1998). El modelo de los "**sesgos pre-existentes"** ("pre-existing bias"; Basolo 1990, 1995a, b), enfatiza la evolución de nuevas señales como consecuencia de los sesgos ya presentes en el receptor, mientras que el modelo de "**explotación sensorial**" ("sensory explotation"; Ryan y Rand 1993a) enfatiza la modificación evolutiva de las señales en respuesta a las características del sistema sensorial del receptor. El modelo de "**trampas sensoriales**" ("sensory traps"; West-Eberhard 1984; Christy 1995) hace referencia al "reciclado" de funciones sensoriales desde contextos diferentes al de la comunicación sexual. De similar modo, Guilford y Dawkins (1991) sugirieron que la evolución de las señales y las preferencias se ven influidas por procesos cerebrales de procesado de la información ("**psicología del receptor**"; "receiver psicology"). Los sesgos evolutivos así generados se han denominado "**impulsos preceptivos**" ("perceptual drive"; Hurd et al. 1995), siendo, en realidad, ya conocidos en la etología clásica (Alexander 1962; Barlow 1977), pero sólo recientemente observados en el contexto de la comunicación sexual (Lande 1981; Hurd et al. 1995). Así, la forma de algunas señales puede depender de las características del sistema sensorial del receptor, derivada de anteriores estados evolutivos, el azar o adaptaciones a otros contextos (Endler y Basolo 1998) ■

ECOLOGÍA Y EVOLUCIÓN DE LAS SEÑALES SEXUALES

La variación natural de las señales y los receptores de éstas, tanto entre especies o poblaciones, como dentro de ellas, proporciona una forma de examinar qué factores contribuyen a la evolución de los sistemas de comunicación. Las señales son rasgos o comportamientos adaptativos que aparecen por mediación de la selección natural ejercida por numerosos factores (Wilson 1975; Endler 1993). En primer lugar, diversos factores ecológicos pueden tener efectos directos en la variación de las señales entre poblaciones o entre especies, impulsando la evolución de los sistemas de comunicación. Las señales deben ser transmitidas a través del ambiente, por lo que las características de éste, tanto bióticas como abióticas, impondrán una selección sobre el sistema comunicativo (por ej. Endler 1991; Fleishman 1992). Además, el ambiente puede afectar indirectamente a las señales comunicativas al ejercer una presión selectiva sobre rasgos morfológicos correlacionados con algún aspecto del sistema de comunicación (Ryan 1988; Ballentine 2006). El ejemplo más obvio es el tamaño corporal, que puede depender de diversos factores y afectar a la extensión y número de señales visuales (Ord y Blumstein 2002), o a la frecuencia de las señales acústicas (Nevo y Capranica 1985), funcionando, a su vez, como índice.

En segundo lugar, las señales se encuentran sometidas a presiones selectivas diferentes a las de otros rasgos debido a la doble interacción entre los receptores y emisores de éstas. Las características del receptor de la señal ejercen presiones selectivas sobre el diseño de la señal, favoreciendo a los emisores que consiguen respuestas favorables. Al mismo tiempo, el diseño de la señal ejerce una presión selectiva recíproca sobre el comportamiento del receptor, favoreciendo a aquellos que interpreten con más precisión la naturaleza e intenciones del emisor (Johnstone 1997; Endler 1992, 1993).

Para examinar los diferentes factores que afectan a la evolución de las señales, podemos considerar tres aspectos: el **diseño estructural (forma de la señal)**, del que dependerá la eficacia de la señal; el **diseño funcional**, del que dependerá el contenido de información de la señal; y el **diseño estratégico**, del que dependerá la fiabilidad y honestidad de la señal. Junto con las restricciones o inercias impuestas por la historia filogenética, la influencia de diferentes factores ambientales sobre cada uno de los tres aspectos, que a continuación detallamos, determinarán el diseño final y el número de señales que usa cada especie.

Diseño estructural: la efectividad de la señal

El diseño estructural o la forma de las señales, tanto intrasexuales, como de elección de pareja, se verá afectado por las características abióticas del ambiente (las características del hábitat, la humedad, temperatura, etc.) y por algunas características bióticas (depredadores y parásitos). Los mecanismos de recepción de la información evolucionan para incrementar la eficiencia y fiabilidad de esa recepción, mientras que las señales evolucionan para aumentar la eficiencia de la comunicación y la manipulación del receptor para beneficio del emisor (Endler 1993). Así, los intereses del emisor y del receptor no tienen por qué coincidir, ni dentro de la misma especie (Dawkins y Krebs 1978; Guilford y Dawkins 1991), pero la recepción de la señal debe ser lo suficientemente clara para permitir al receptor tomar una decisión comportamental o fisiológica. La eficacia depende, por un lado, de los factores que afectan a la emisión de la señal y al emisor, y por otro, de aquellos que afectan a la recepción de la señal y al receptor (Endler 1993). Además, también depende del sistema sensorial usado en cada contexto o especie, lo cual puede derivarse de la historia evolutiva (Ord y Stuart-Fox 2006) o de la adaptación al medio (Podos 2001; Montealegre-Z 2008), en función de las distintas ventajas de cada modalidad sensorial (ver Tabla 1).

Tabla 1. Ventajas e inconvenientes de los principales tipos de señales (modificado de Endler 1993).

Tipo de señal	Ventajas	Inconvenientes
Visual	-Muy rápidas -Muchos componentes -Transferencia de información elevada	-Dependen de la luz ambiental -Contacto visual necesario
Auditiva	-Muy rápidas -Algunos componentes -Transferencia de información media	-Ruido ambiental -No pueden ocultarse ante los depredadores o parásitos
Química (olfativa)	-Muchos componentes -Muy específicas -Relacionadas directamente con la fisiología -Buenas indicadoras de fitness	-Lentas -Necesarios receptores muy específicos -Poco controlable en cuanto a emisión o rastreo

1. El efecto del ambiente abiótico

Tanto las características físicas (temperatura, humedad, estructura, luminosidad, etc), como bióticas (depredación, parasitismo) pueden afectar a la efectividad con que es emitida y recibida la señal, imponiendo una selección de la forma de la señal a través de un proceso conocido como "impulso sensorial" ("Sensory drive" Endler 1992), así como del número de señales utilizadas (ver *Comunicación Multisensorial y Señales Múltiples; Capítulo 1*). Las señales evolucionan para minimizar los efectos del "ruido ambiental" (entendido como cualquier factor que distorsione la señal) o la interferencia de las señales de otras especies (Bradbury y Vehrencamp 1998). Así, la selección favorecerá las señales que experimenten la menor atenuación y degradación por parte del medio a través del que se transmitan (Endler 1992; 1993). La intensidad de la señal decrece con la distancia de distinta manera dependiendo de los distintos componentes de la señal (olfativa o química) o de las diferentes frecuencias (en señales visuales, auditivas o eléctricas) (Bradbury y Vehrencamp 1998). Algunas de estas distorsiones de la señal pueden depender de factores como la temperatura o la humedad (Alberts 1992), o de la heterogeneidad del hábitat. Por ejemplo, estudios sobre señales acústicas han demostrado su adaptación a las condiciones ambientales locales para aumentar la efectividad de transmisión (Ryan et al. 1990; Boncoraglio y Saino 2007). En sistemas de comunicación visual, variaciones en la heterogeneidad, luz o, en ambientes acuáticos, claridad de transmisión ambiental, también pueden influenciar la efectividad de las señales (Fleishman 1992; Gomez y Théry 2004; Endler et al. 2005). Por lo tanto, estudios interespecíficos (por ej., Marchetti 1993) e intraespecíficos (por ej., Endler y Houde 1995) sobre variación de las señales sugieren que la variación geográfica de las características del hábitat puede afectar a la evolución de las señales. Además, el ambiente puede afectar también al comportamiento de señalización, puesto que los animales que escojan el lugar más adecuado (que optimice su efectividad) para transmitir sus señales serán favorecidos (Endler y Théry 1996). Por lo tanto, cualquier estudio sobre evolución y diseño de señales sexuales debería considerar los factores ambientales que afectan a la calidad de la recepción y procesamiento de la señal.

2. El efecto del ambiente biótico

La depredación también impondrá unos costes al emisor de la señal que, indirectamente pueden condicionar la efectividad de ésta (pero ver *Capítulos 2* y *3*). Por ejemplo, Endler (1980) demostró que la presencia de depredadores visuales en algunas áreas provocaba una fuerte presión selectiva sobre el patrón de coloración que los machos de guppy

Imagen 2. Algunos depredadores se especializan en explotar determinadas señales sexuales de sus presas, ejerciendo una presión selectiva sobre éstas o sobre el comportamiento asociado a la señalización. Las culebras lisas (*Coronella austriaca*) son depredadores saurófagos que utilizan las señales sexuales químicas de las lagartijas serranas para localizarlas y atrapalas (Amo et al 2004; *Capítulo 3*).

utilizaban para atraer a las hembras. Del mismo modo las variaciones en el canto que presentan algunas especies de rana pueden ser explicadas por la presión que ejerce la presencia de murciélagos. Estos depredadores utilizan algunas características del canto relacionadas con la elección de pareja para localizarlas y cazarlas (Ryan et al. 1982). Sin embargo, el comportamiento también puede variar con las presiones que la depredación ejerce sobre la evolución y uso de las señales aumentando la exposición de éstas, o reduciéndola, en el caso de comportamientos compensatorios (Hedrick 2000; *Capítulos 2* y *3*).

3. Los sesgos del receptor

El procesado de la información ("**psicología del receptor**"; "receiver psicology") puede presentar sesgos derivados de las funciones cerebrales (Guilford y Dawkins 1991; Rowe 1999), denominados "**impulsos preceptivos**" ("perceptual drive"; Hurd et al. 1995). Estos sesgos pueden conducir las formas de las señales para que estas sean más efectivas, bien por explotación de los emisores de las tendencias existentes, bien por coevolución de las señales a medida que evoluciona el sistema sensorial del receptor (ver *La Comunicación Sexual: señales no indicadoras*). La adaptación a otros aspectos ajenos a la comunicación también puede condicionar la emisión de señales (por ej., Podos 2001; Montealegre-Z 2008).

Diseño funcional: información e intereses

El diseño funcional hace referencia al contenido de información de las diferentes señales sexuales. En general, el contenido de información de las señales es muy variable y depende de múltiples factores (Dawkins y Guilford 1991). En primer lugar, en función de si las señales son informativas o persuasivas, se generarán diferentes escenarios evolutivos, favoreciéndose "carreras de armamentos" simétricas (emisor y receptor mejoran progresivamente la transmisión de la información) o asimétricas (el emisor genera señales más persuasivas pero el receptor aumenta sus resistencia a ser manipulado), respectivamente (Berglund et al. 1996). Por otro lado, las señales sexuales pueden ser usadas en procesos de selección sexual intrasexual o de elección de pareja, aunque a menudo las señales son "útiles" en ambos contextos (Qvarnström y Forsgren 1998; Wong y Candolin 2005). Las señales usadas en contextos de competencia intrasexual informan sobre el estatus de dominancia del emisor (Kim 1995; Martín et al. 2007), es decir, su agresividad o habilidad de lucha, mientras que las señales usadas para la elección de pareja informan sobre diferentes características "beneficiosas" del emisor o bien de su localización (Andersson 1994; ver *Comunicación Sexual*). El contenido concreto de información puede ser muy variado (Dawkins y Guilford 1991) y dependerá de las características ambientales (por ej., Welch 2003). Así, entre especies, la mayor o menor competencia intrasexual puede determinar la complejidad de las señales (Ord et al. 2001) o su mayor o menor desarrollo (Kodric-Brown 2003). Del mismo modo, las características sociales determinarán si las señales informan sobre el estatus de dominancia de los machos o de otros factores (Qvarnström y Forsgren 1998; Wong y Candolin 2005; *Capítulo 4*). Otros factores ecológicos, como la mayor o menor presencia de parásitos también podrían favorecer el mayor o menor contenido de información respecto al estado de salud (Boughman 2007).

Imagen 3. Las distribución de los recursos o de las hembras determina la organización social de las especies, de forma que la selección natural puede haber favorecido comportamientos como la territorialidad o “guardar” a las hembras, como parece ser el caso del macho de lagartija cantábrica (*Iberolacerta cyreni*) de la imagen; o bien, la búsqueda activa de hembras, como hace la lagartija carpetana (*I. cyreni*) (Salvador et al. 2008; ver Capítulo 4).

Diseño estratégico: la “honestidad” de la señal

El diseño estratégico hace referencia a como la señal es favorecida por la selección natural para asegurar su “honestidad” (Maynard-Smith y Harper 2003). Aunque el “engaño” ocasional no tiene por qué interrumpir el sistema de señalización, ya que las señales son, en general, honestas (Johnstone y Grafen 1993) dependiendo de las diferencias ecológicas, el mecanismo que les confiere honestidad podrá variar. Por ejemplo, las interacciones sociales son un “coste” que confiere honestidad a algunas señales (Rohwer 1982) y que pueden variar en función de las diferencias en el sistema social o las estrategias de apareamiento (Berglund et al. 1996; Wong y Candolin 2005; *Capítulo 4*). El reparto de los recursos o de las hembras puede influir sobre los beneficios de la territorialidad (Emlen y Oring 1977; Arnold y Duvall 1994) y, por tanto, sobre la importancia y el número de las interacciones sociales que determinarán la honestidad de las señales implicadas. La depredación también puede ser un coste de ciertas señales (Zuk y Kolluru 1998), por lo que variaciones en la presión depredadora alterarán el mecanismo que les confiere honestidad (Ryan et al. 1982). Finalmente, los elevados niveles de andrógenos necesarios para producir y mantener algunas señales tienen efectos negativos sobre el sistema inmune o sobre la condición física (por ej., Salvador et al. 1996; Olsson et al. 2000). El equilibrio necesario en la asignación de recursos (Mougeot et al. 2009) puede variar en función de las condiciones ambientales. Por ejemplo, un mayor riesgo de parasitación o menos recursos alterarán la relación de dependencia y promoverán diferencias en la honestidad relativa de las señales entre poblaciones o especies (Boughman 2007; Judge et al. 2008) ■

COMUNICACIÓN MULTISENSORIAL Y SEÑALES MÚLTIPLES

Aunque la mayoría de los trabajos sobre selección sexual se centran en una sola señal (o interpretan como una señal simple lo que podría ser una señal múltiple), lo cierto es que muchos animales producen y responden a señales múltiples (Rowe 1999; Candolin 2003). Por lo tanto, para entender bien cómo funcionan los sistemas de comunicación o selección sexual, debería tenerse presente el conjunto de las distintas señales sexuales utilizadas y su interacción.

Las señales múltiples han sido descritas como **señales con múltiples componentes** ("multicomponent signals"; Hölldobler 1995; Johnstone 1995), cuando se refieren a un solo sistema sensorial, o **señales multisensoriales** ("multimodal signals"; Guilford y Dawkins 1991; Rowe y Guilford 1999), cuando sus componentes pertenecen a más de una modalidad sensorial. El uso tanto de unas como de otras podría ser adaptativo (por ejemplo al incrementar la eficacia biológica reduciendo los errores o los costes de elección, ver abajo). Alternativamente, podría deberse a remanentes de criterios de selección pasados (Ryan y Rand 1993a), o que surgieron en otro contexto, y que han sido explotados por el emisor aprovechando la existencia de esos favoritismos sensoriales ("sensory bias") preexistentes (Candolin 2003). Lo cierto es que existen diversas hipótesis no excluyentes que podrían explicar el origen adaptativo de diferentes casos de señalización múltiple (Candolin 2003).

La hipótesis de los **múltiples mensajes** ("multiple messages hypothesis") sugiere que diferentes señales o componentes de una señal pueden proporcionar información sobre diferentes cualidades del individuo (Møller y Pomiankowski 1993; Johnstone 1997). Así, las señales o sus componentes podrían ser evaluados juntos, indicando la calidad general del individuo, o alternativamente, de manera que diferentes receptores prestarán atención a diferentes señales o componentes de estas y, por lo tanto a diferentes cualidades del emisor, de acuerdo a su propia condición o configuración genética (Wedekind 1992). En este sentido varios estudios han encontrado que diferentes ornamentos reflejan diferentes aspectos de la calidad de pareja. Por ejemplo, las coloraciones compuestas a partir de distintos pigmentos a menudo reflejan diferentes cualidades; los carotenoides suelen señalizar la condición física, especialmente el estado de salud (revisado por Møller et al. 2000), mientras que los colores basados en melaninas, sobre todo señalizan el estatus social (revisado en Senar 1999; pero ver Fitze y Richner 2002). En conjunto, muchos estudios sugieren que rasgos que tradicionalmente son evaluados como uno solo, como la coloración, la calidad del canto o el comportamiento de cortejo, pueden consistir en varios componentes. Cada uno de esos componentes porta su propio mensaje sobre algún aspecto de la calidad del emisor y proporcionan una señalización más clara de la calidad de pareja cuando son evaluados juntos por el receptor (Wedekind 1992). Otra posibilidad es que diferentes ornamentos indiquen la condición del individuo a lo largo de diferentes escalas de tiempo, con unas señales reflejando la condición a lo largo de una escala de tiempo amplia, mientras otras son más dinámicas y responden a cambios rápidos de la condición (por ej., Sorenson y Derrickson 1994; Hill et al. 1999). En este sentido, una hipótesis no verificada, es que los ornamentos que se desarrollan en largos periodos de tiempo, son buenos indicadores de calidad genética, mientras que la señales más flexibles que reflejan la condición del momento, serían mejores indicadoras de beneficios directos, como la habilidad parental o la capacidad de fertilización (Candolin 2003). La divergencia de criterios entre las hembras de distintas especies ha sido puesta de manifiesto en numerosas ocasiones (por ej., Jennions y Petrie 1997; Widemo y Saether 1999; Tregenza y Wedell 2000). Sin embargo, la posibilidad de que las hembras difieran en la atención que prestan a diferentes aspectos cualitativos de los machos y, consecuentemente, difieran en cómo sopesan las diferentes señales, ha recibido relativamente poca atención (pero ver Soler et al. 1998). Finalmente, unos pocos estudios han mostrado que variaciones en el ambiente biológico, como el riesgo de depredación o la jerarquía de dominancia, pueden afectar las preferencias por múltiples señales (Reid y Weatherhead 1990; Kodric-Brown 1993; Endler y Houde 1995; Marchetti 1998). Por ejemplo, en el caso del guppy, las hembras prestan menos atención a los ornamentos masculinos cuando las interacciones agonísticas entre machos son más frecuentes, mientras que prestan más atención a los rasgos comportamentales que indican dominancia (Kodric-Brown 1993). En cualquiera de los casos, la hipótesis de los múltiples mensajes supone un valor adaptativo al uso de señales múltiples.

La hipótesis de las **señales de refuerzo o redundantes** (“back-up o redundant signal hypothesis”) propone que múltiples señales permiten una evaluación más precisa del la calidad de pareja al reflejar cada señal la misma calidad con algún error (Møller y Pomiankowski 1993; Johnstone 1997). Esta hipótesis difiere de la anterior en que las diferentes señales reflejan el mismo aspecto de la calidad del individuo, en lugar de diferentes aspectos o cualidades. El uso de señales de refuerzo o redundantes podría reducir los errores de elección de pareja o, alternativamente, el tiempo y la energía gastados en inspeccionar las posibles parejas si las señales de refuerzo facilitan la comparación entre los diferentes individuos. Además, este tipo de señales dificultaría la aparición de “tramposos” (“cheaters”) que señalizasen falsamente su calidad. Se supone que existe cierto grado de deshonestidad en los sistemas de comunicación estables (Dawkins y Guilford 1991; Johnstone y Grafen, 1993; Johnstone 1994), promovido, por ejemplo, por diferencias individuales en el coste de las señales (Kokko 1998; Candolin 1999, 2000; Backwell et al. 2000). Sin embargo, los diferentes costes impuestos por las diferentes señales o el coste total de expresar múltiples señales aseguraría la honestidad de todas ellas (por ej., Bortolotti et al. 2006), evitando la expresión de varias señales deshonestas y teniendo también, por tanto, un valor adaptativo. Distinguir qué casos de señalización múltiple se corresponden con la hipótesis de las señales redundantes y cuales con la hipótesis de los múltiples mensajes puede ser difícil, ya que los rasgos que señalizan las diferentes características (mensajes múltiples) pueden estar correlacionados con la calidad general del individuo y funcionar, por tanto, como señales redundantes de esa calidad (por ej., Price 1984; Kvarnemo 1995;

Bart y Earnst 1999; Candolin y Voigt 2001). Por ejemplo, algunos estudios muestran una correlación entre la calidad fenotípica del los machos y las características de sus territorios, pudiendo considerarse ambos como señales redundantes de la calidad genética de aquellos (Balmford et al. 1992). Sin embargo, lo contrario también ocurre (por ej., Carranza 1995; Buchanan y Catchpole 1997; Goulet 1998; Soler et al. 1998), indicando que una correlación entre los distintos rasgos o señales no debe ser el patrón general. De hecho, la mayor parte de los estudios muestran que no existe correlación entre las señales múltiples (por ej., Birkhead et al. 1998; Marchetti 1998; Badyaev et al. 2001; pero ver Badyaev et al. 1998), lo que sugiere que las señales redundantes son menos comunes que los mensajes múltiples (Candolin 2003).

Imagen 4. Aunque la mayor parte de los estudios sobre señales sexuales suelen centrarse en las más llamativas, lo cierto es que la mayor parte de los animales utilizan múltiples señales comunicativas, a menudo de más de un sistema sensorial. Por ejemplo, las arañas utilizan señales visuales, pero también táctiles o químicas (Rypstra et al. 2009).

En contraposición a los dos modelos anteriores, que suponen un valor indicativo a las señales, la hipótesis de las **señales poco fiables o de Fisher** (“unreliable cues”) sugiere que existen múltiples preferencias debido a los sesgos del receptor. Estos sesgos, consecuencia del pasado evolutivo (Ryan y Rand 1993a; Holland y Rice 1998), se acumularían al ser explotados por los emisores. Así, aunque no proporcionen más información sobre la calidad del emisor, al contrario de lo que sugieren algunos autores (Candolin 2003), también aumentarían su eficacia biológica a través de un proceso de selección desbocada (“runaway”) o facilitando la detección del individuo (Møller y Pomiankowski 1993). En este contexto, se ha encontrado que el tamaño de los ornamentos está relacionado negativamente con la asimetría en especies con señales simples, pero no en especies con señales múltiples, indicando que sólo las señales simples suelen ser dependientes de la condición (Møller y Pomiankowski 1993). Prum (1997) también encontró que el patrón evolutivo de las señales de un linaje de aves era consistente con las predicciones de la hipótesis de las señales fisherianas, al ser el repertorio diverso, jerárquico y compuesto por señales que han evolucionado de forma independiente en diferentes épocas a lo largo de la historia evolutiva del linaje. Sin embargo, la frecuente observación de múltiples señales indicando algún aspecto de la calidad del emisor refuta la asunción de que la mayor parte de las señales no son informativas. Aunque esto podría ser debido a un sesgo en la publicación de estudios al respecto, la calidad o cualidad que indica una determinada señal puede ser difícil de identificar o medir, por lo que muchas señales indicadoras podrían ser clasificadas como no informativas (Candolin 2003).

Similar a la anterior, la hipótesis de las "**señales de umbral**" ("threshold signals"; Holland y Rice 1998) se basa en que los intereses de ambos sexos a menudo difieren debido a las diferencias en su potencial reproductivo (Bateman 1948; Trivers 1972). Las hembras, con menor potencial reproductivo, suelen beneficiarse de ser más selectivas y la aparición de señales que explotasen sesgos sensoriales preexistentes ayudaría a los machos a vencer su resistencia a aparearse. A su vez, las hembras que desarrollasen mayor resistencia a "sucumbir" ante las señales masculinas serían favorecidas por la selección natural, estableciéndose una "carrera de armamentos asimétrica" (Holland y Rice 1998). Así, estarían evolucionando continuamente nuevas señales masculinas que se añadirían a las que, aunque ya no son útiles para vencer la resistencia femenina, aún son necesarias para conseguir la atención de las hembras (superar el "umbral"), antes de que éstas se "fijen" en los nuevos rasgos (Holland y Rice 1998).

Tampoco supone necesariamente una función indicadora la que podríamos llamar hipótesis de las "**señales aditivas**", que destaca el efecto de la psicología del receptor, sugiriendo que varias señales provocarían una mayor respuesta del receptor que las señales aisladas (Guilford y Dawkins 1991, 1993; Rowe 1999). Así, los emisores que produjesen señales múltiples aumentarían sus posibilidades de ser detectados, reconocidos, discriminados y memorizados (por ej., Hultsch et al. 1999; Rowe 2002). Aunque la mayoría de los ejemplos de señales aditivas se refieren a señales no sexuales (por ej., aposematismo; Siddall y Marples 2008) probablemente los mismos beneficios de la multiplicidad de estas señales pueden aplicarse a las señales sexuales (Rowe 1999). Así, algunos componentes de una señal que no contienen información, suponen un estímulo accesorio que realiza una función psicológica. Por ejemplo, el movimiento puede aumentar la detectabilidad de las señales visuales (Fleishman 1988; Endler y Théry 1996) y algunas manchas pueden amplificar otras señales sin ser el centro del interés de la selección (Hasson 1991; Moya-Laraño et al 2003; Galván y Sanz 2008). Esta interacción entre componentes, que puede ocurrir tanto dentro de un mismo sistema sensorial (Cohen 1997; Aydin y Pearce 1997) como entre sistemas sensoriales, implica que las señales múltiples no pueden ser completamente comprendidas estudiándolas de forma aislada.

La hipótesis de los "**múltiples ambientes sensoriales"** incide sobre el hecho de que la habilidad para detectar y examinar las señales varía en función de las condiciones ambientales o la distancia, por lo que la selección natural favorecería a aquellos individuos que utilizasen diferentes señales bajo diferentes condiciones. Esto facilitaría la optimización de la eficacia de cada señal respecto a las condiciones bajo las que es utilizada, reduciendo los costes de elección. Diferentes señales de un mismo sistema sensorial pueden presentar distintas ventajas dependiendo del medio (por ej., Endler y Houde 1995), pero señales de distintas modalidades sensoriales tienen diferentes ventajas (ver Tabla 1).

Además, las distintas señales podrían proporcionar la misma información (señales redundantes) o diferente (múltiples mensajes). Por ejemplo, algunas señales podrían aumentar la detección a larga distancia, mientras otras reflejan la calidad individual a corta distancia (López y Martín 2001). Muchos trabajos han encontrado que diferentes señales son utilizadas a distintas distancias (Backwell y Passmore 1996; Gibson 1996; Candolin y Reynolds 2001; Suk y Choe 2002), en diferentes etapas del cortejo (Shine y Mason 2001; Luttbeg et al. 2001) o en diferentes hábitats (Reynolds 1993; Endler y Houde 1995). La mayoría de estos trabajos encuentra que las señales múltiples aumentan la cantidad de información transmitida (por ej., Candolin y Reynolds 2001) o facilitan su examen bajo diferentes condiciones (Reynolds 1993; Endler y Houde 1995). Por ejemplo, Shine y Mason (2001) encontraron que los machos de jarretera, *Thamnophis sirtalis parietalis*, utilizan señales visuales o térmicas mientras buscan hembras, pero las señales químicas adquieren mayor importancia para decidir qué cortejar, una vez se ha formado un "acúmulo reproductivo" alrededor de una hembra.

Imagen 5. Los machos de lagartija carpetana (*Iberolacerta cyreni*) presentan tanto señales visuales (ocelos ventrales azules), como señales químicas (secreciones femorales). El uso de cada tipo de señal varía entre poblaciones en función de distintos factores ambientales, como la estructura del microhábitat o la competencia intrasexual (Capítulo 1).

Finalmente, se ha demostrado que algunas especies utilizan diferentes señales para el reconocimiento específico y para la elección de pareja (revisado en Gerhardt 2001; pero ver *Señales y Especiación*). Por ejemplo, en anuros, el ratio de pulso es utilizado para el reconocimiento de especies, mientras que la duración del canto refleja la calidad genética del macho y es utilizada en la elección de pareja (Welchet al. 1998; Bosch y Márquez 1996). La selección sexual y el reconocimiento específico pueden entrar en conflicto cuando las señales utilizadas para elegir pareja coinciden con señales de otras especies simpátricas (Rand et al. 1992; Ryan y Rand 1993b; Pfennig 1998, 2000; Hankinson y Morris 2002). En ese caso, la evolución de señales adicionales para prevenir la hibridación, se verá favorecida (Crapon de Caprona y Ryan 1990; Hankison y Morris 2003) ■

SEÑALES Y ESPECIACIÓN

La selección sexual y el reconocimiento de especies pueden ser considerados como diferentes aspectos de un solo fenómeno de comunicación animal, en el cual la selección sexual puede acabar convirtiéndose en reconocimiento específico (Ryan y Rand 1993b; Andersson 1994). Así, el aislamiento comportamental (o aislamiento precópula) entre especies puede aparecer como consecuencia de los procesos de selección sexual dentro de cada especie (Lande, 1981; Lande y Kirkpatrick, 1988; Turner y Burrow, 1995; pero ver Bennett y Owens 2002; Hankison y Morris 2002). Las diferentes condiciones ambientales de distintas poblaciones podrían, así, acabar convirtiéndose en un proceso de especiación ecológica impulsado por la adaptación de las señales a las distintas condiciones (revisado en Boughman 2002). Además, el aprendizaje de determinadas señales también puede jugar un papel importante en los procesos de especiación al originar dialectos locales que generan un aislamiento reproductor (Slabbekoorn y Smith 2002).

La mayor parte de los estudios sobre especiación sexual se han basado en la divergencia de las señales visuales de diferentes especies de peces. Por ejemplo, en peces de arrecifes o guppies, el uso de distintas señales visuales debido a distintas presiones ambientales tiene como consecuencia un proceso de especiación (Grether et al. 2001; Boughman 2002; Puebla et al. 2007). La divergencia en las características del receptor ("psicología del receptor") también se ha demostrado que pueden determinar la evolución paralela de distintas especies o géneros de peces (por ej., Boughman 2002, Simmons et al. 2001; Smith et al. 2004). Finalmente, las señales químicas juegan un importante papel en el reconocimiento de especies (Phelan y Baker 1987; revisado en Smadja y Butlin 2009) y diversos estudios demuestran la implicación de su divergencia en procesos de especiación de diversos grupos, como insectos (Bengtsson y Löfstedt 2007), peces (Plenderleith et al. 2005) o anfibios (Buckley et al. 2005). En reptiles, se ha demostrado que la adaptación de las señales visuales a las distintas condiciones lumínicas de los hábitats ocupados por distintas especies del género *Anolis* puede impulsar su especiación (Leal y Fleishman 2002; Macedonia 2001). Sin embargo, aunque se sabe que las señales químicas también están implicadas en su reconocimiento específico (Cooper y Vitt 1987; LeMaster y Mason 2003; Shine et al. 2002), apenas se ha estudiado su implicación en los procesos de especiación (pero ver Martín y López 2006a,b).

Por otro lado, en especies simpátricas, los procesos de refuerzo ("reinforment") para evitar la hibridación también impulsarán la evolución de señales exclusivas de reconocimiento de especie diferentes de las señales sexuales (por ej., Hankison y Morris 2003). Debido a su especificidad en cuanto a función, estas señales serán menos variables entre individuos que las implicadas en la selección sexual (Endler et al. 2005; Zuk et al. 2008) ■

LA SEÑALIZACIÓN SEXUAL EN REPTILES Y LA PSICOLOGÍA DEL INVESTIGADOR

Los investigadores, al igual que los depredadores, son también animales que se basan principalmente en una o varias modalidades sensoriales para adquirir información del medio, de los individuos coespecíficos o de sus “presas”, en este caso, sus objetos de estudio. Esto introduce inevitablemente un sesgo de apreciación en cuanto a lo estudiado, que en el caso del hombre es visual. De este modo, tradicionalmente, la mayor parte de los estudios de selección sexual se han realizado con señales visuales en grupos animales especialmente basados en esa modalidad sensorial; es decir, aves y, en menor medida, peces. Del mismo modo, se ha considerado que los reptiles son un grupo principalmente visual, debido a la facilidad relativa con la que se observan sus señales visuales, en contraposición a otro tipo de señales. Sin embargo, aunque las señales visuales son importantes en todos los aspectos de su ciclo vital (Carpenter y Ferguson 1977), sólo recientemente se ha puesto de manifiesto la importancia de la comunicación química (Mason 1992). Aunque el uso diferencial de una u otra modalidad sensorial varía dependiendo del grupo, debido a diferentes aspectos de su biología o su filogenia (Cooper 1994), la mayor parte de las especies de reptiles utilizan múltiples señales, tanto visuales como químicas. Muchas especies de lagartos muestran, durante sus interacciones sociales, dramáticos cambios de color y brillantes patrones, muy conspicuos al observador humano. La mayoría de estas especies son diurnas y habitantes de medios abiertos, debido a su dependencia del sol para la termorregulación, por lo que la señalización visual resulta muy adecuada (Gans y Crews 1992). No obstante, la comunicación química también suele estar presente en muchos de ellos, probablemente debido a las diferentes ventajas de cada modalidad sensorial (Mason 1992; Bradbury y Vehrencamp 1998; ver Tabla 1). Otros grupos, habitantes de medios menos abiertos o nocturnos (como ofidios, anfisbénidos o cocodrilos) se basan más en la comunicación química, o incluso acústica (excepcional entre los reptiles, pero presente en cocodrilos o algunos gekónidos). Los rasgos comportamentales o morfológicos evolucionados para otros propósitos, también pueden condicionar la comunicación sexual de los reptiles. Por ejemplo, los diferentes sistemas de búsqueda o caza del alimento, es decir, los rastros o indicaciones (“cues”) utilizados para localizar presas o alimento, pueden impulsar el uso de una determinada modalidad sensorial (Cooper 1994). La mayor parte de los lagartos son cazadores al acecho, que utilizan la vista para localizar a sus presas, por lo que el uso de comunicación sexual visual estará, lógicamente favorecido (Gans y Crews 1992). Otras especies (como muchos ofidios) son buscadores activos de presas, basados, sobre todo en señales químicas (cairomonas), debido a la facilidad de su explotación (Zuk y Kolluru 1998; ver Imagen 2). Incluso en algunos grupos, como los camaleones, las adaptaciones especiales para la captura de presas (lengua protráctil) impiden su uso para la comunicación química, restringiendo ésta a la señalización visual, la cual es más sofisticada (Stuart-Fox et al. 2007).

Comunicación visual en reptiles

Las señales visuales de los reptiles pueden ser movimientos, como los "cabeceos" o extensiones gulares de algunos iguánidos, o el "pataleo" de los lacértidos; o bien patrones y cambios de coloración muy diversos. Además, como otros muchos grupos, los reptiles pueden ver en el espectro ultravioleta y, a menudo sus señales lo reflejan (Arribas 2001; Thorpe y Richard 2001; Macedonia 2001). Las señales visuales se ven fuertemente influidas por el tipo y cantidad de luz que reflejan, la cual dependerá de las características estructurales del hábitat (Endler 1992). Por lo tanto, el tipo de hábitat en el que son desplegadas determinará la coloración y extensión de éstas. Por ejemplo, numerosos estudios (Fleishman et al. 1993; Fleishman y Persons 2001; Leal y Fleishman 2002; Macedonia 2001) han encontrado que la coloración del pliegue gular de diferentes especies del género *Anolis* es la más eficaz dependiendo de la luminosidad del hábitat. Otros trabajos muestran cómo la complejidad del hábitat o la organización social puede explicar las diferencias de coloración entre diversas especies de iguánidos (Baird et al. 1997; Ord et al. 2001, 2002) o camaleones (Stuart-Fox et al. 2007).

Por otra parte, se ha demostrado que estas coloraciones u otros rasgos (por ej., la longitud de la cola) funcionan como señales de estatus (Thompson and Moore 1991b; Olsson 1994; Martín y Forsman 1999). Por ejemplo, los lagartos ágiles (*Lacerta agilis*) desarrollan durante el periodo de celo coloraciones verdes en los flancos cuya extensión está correlacionada con su capacidad de lucha (Olsson 1994 a,b,c) y su estado de salud (Olsson et al. 2005), pero a su vez tiene costes en cuanto a depredación (Olsson 1993). Las señales visuales de algunas especies de lagartos herbívoros (*Sauromalus obesus*) también se correlacionan con su estado nutricional (y por tanto, la calidad del hábitat que defienden frente a otros machos), variando esa relación en función de las diferentes condiciones de cada población (Kwiatkowski y Sullivan 2002).

Imagen 6. Los machos de muchos reptiles desarrollan coloraciones conspicuas durante el celo que median las interacciones agonísticas. Los machos de lagarto verdinegro (*Lacerta schreiberi*) desarrollan coloraciones azules en la cabeza.

Recientemente se ha puesto de manifiesto que la señalización, mediante diferentes coloraciones, de estrategias de apareamiento alternativas (Sinervo et al. 2006) podría ser un fenómeno relativamente común (Vercken et al. 2007; Healey et al. 2008). Sin embargo, en el caso de los reptiles, al contrario que en otros grupos, como aves o peces, la elección de pareja mediante señales visuales parece ser bastante rara (Olson y Madsen 1995; Tokarz 1995).

Comunicación química en reptiles

Aunque la mayor parte de las investigaciones sobre selección sexual en reptiles se han llevado a cabo con señales visuales, algunas evidencias sugieren que las señales químicas o feromonas podrían ser también muy importantes en algunos grupos. Por ejemplo, los ofidios basan su comunicación sexual en las señales químicas que secretan a través de la piel (Gans y Crews 1992) y los machos de numerosas especies de lagartos poseen glándulas epidérmicas femorales que secretan feromonas que intervienen en diversos procesos de selección sexual (Mason 1992; Alberts 1993). Las señales químicas podrían ser determinantes en algunos aspectos importantes de la comunicación social, como el reconocimiento específico (Cooper y Vitt 1987; LeMaster y Mason 2003; Shine et al. 2002), la identificación sexual (López y Martín 2001b; López et al 2002a; Gonzalo et al. 2004), el parentesco (Bull et al. 2001) o el reconocimiento individual (Alberts y Werner 1993; Aragón et al. 2000; 2001b). Además, recientemente diversos trabajos han puesto de manifiesto la importancia de las señales químicas en la comunicación sexual de ofidios (Shine et al 2003) o lacértidos. Dentro de estos últimos se ha demostrado la implicación de las señales sexuales en los procesos de competencia entre machos (López y Martín 2001, 2002; López et al. 2002; ver *Especies de Estudio: Las Lagartijas Serranas*) o en la selección de pareja por parte de las hembras en cuanto a diversas características de los machos, como edad, simetría o sistema inmune (López y Martín 2005; Martín y López 2006; Martín et al. 2007; ver *Especies de Estudio: Las Lagartijas Serranas*) ■

Imagen 7. Algunos grupos de reptiles basan su comunicación sexual en las señales químicas o feromonas, como es el caso de la víbora hocicuda (*Vipera latastei*).

ESPECIES DE ESTUDIO: LAS LAGARTIJAS SERRANAS

Las lagartijas serranas (*Iberolacerta* sp.) son un grupo de 7 especies de lacértidos, exclusivos de la península ibérica, con una distribución alopátrica y asociada, en general, a roquedos de alta montaña. Desde un punto de vista zoogeográfico, se ha propuesto que la distribución actual es una consecuencia de una amplia distribución en el pasado, que se vio drásticamente reducida ante la competencia por parte de otras lagartijas del género *Podarcis*. También se postula un origen periglaciar de la especie, pero se desconocen las características biogeográficas anteriores a la distribución actual (Pérez-Mellado 1997).

De la antigua consideración de *Lacerta monticola* como especie única en la Península, las poblaciones del Pirineo, antes consideradas subespecie (*L. m. bonnali*) fueron elevadas a rango específico (*Iberolacerta bonnali*, *I. aurelioi*, *I. aranica*). Del resto de poblaciones, datos genéticos recientes (electroforesis de proteínas y ADN) confirman la diferenciación de tres especies diferentes: la lagartija cantábrica , *Iberolacerta monticola* (Serra de Estrela, en Portugal, Galicia y Cordillera Cantábrica), la lagartija carpetana, *I. cyreni* (Sierras de Guadarrama y Gredos), la lagartija de las batuecas, *I. matinezricai* (Peña de Francia) y la lagartija leonesa, *I. galani* (Montes de León) (Odierna et al. 1995; Arribas 1996, 1999a, b; Mayer y Arribas 1996; Arribas y Carranza 2004; Carranza et al. 2004).

En la presente Tesis Doctoral se realizan diversos estudios con individuos y poblaciones de la Sierra de Guadarrama (Madrid) de lagartija carpetana y con individuos de lagartija cantábrica de una población a nivel del mar en A Serra da Capelada (Galicia) (ver Cuadro 2).

La lagartija carpetana

La lagartija carpetana es una especie típicamente ligada a roquedos de alta montaña con adaptaciones morfológicas para moverse por las rocas y refugiarse en grietas de rocas. En la Sierra de Guadarrama, utiliza canchales de rocas grandes, con matorrales como piornos (*Cytisus oromediterraneus*) y enebro rastrero (*Juniperus communis*), y pastizales de *Festuca* sp. y otras hierbas. A nivel de selección de microhábitat, evita los enebros y los pastizales, y prefiere los roquedos (Martín y Salvador, 1992, 1997a). En Guadarrama se han estimado densidades de población entre 220-328 ind./ha (Martín y Salvador, 1997a). Aunque las poblaciones de *I. cyreni* pueden alcanzar localmente densidades relativamente altas, están estrictamente correlacionadas con la presencia de un microhábitat específico (canchales de rocas) (Martín y Salvador 1992, 1997a; Martín 1992). En la mayoría de las poblaciones aparece entre los 1.600 y 2.000 m. y en Guadarrama aparece por encima de los 1.760 m (Puerto de Cotos, Navacerrada), hasta las cumbres de Peñalara a 2.340 m (García-París et al. 1989a, b).

Organización social y selección sexual

Es una especie poligínica en la que los machos adultos tienen territorios de tamaño variable que tratan de incrementar aumentando su tasa de movimientos para así acceder a un mayor número de hembras que son más sedentarias (Salvador et al. 2008). En Guadarrama y Gredos el tamaño del dominio vital de los machos varía entre 8.5-442 m^2, solapando con una media de 13,7 machos vecinos, mientras que las hembras tienen dominios vitales de entre 2,6-679 m^2, y los subadultos y juveniles entre 1,7 y 170 m^2 (Martín y Salvador 1997b; Aragón et al. 2004). El tamaño del territorio de los machos disminuye al finalizar la época de los apareamientos, debido a una reducción de la actividad y movimientos, y en los machos que han perdido la cola, debido a que reducen sus movimientos a microhábitats más seguros (Aragón et al. 2001a, b). Existe un alto solapamiento entre los dominios vitales de varios machos vecinos y los enfrentamientos agonísticos son frecuentes. Los machos adultos (típicamente con coloración dorsal verdosa-azulada) guardan a las hembras y defienden territorios, pero los más jóvenes (coloración parda) pueden adoptar una estrategia alternativa de satélite, siendo menos conspicuos, sin defender ningún territorio, y robando cópulas en los territorios de otros machos (Salvador et al. 2008). Cuando la densidad de machos es alta pueden formarse jerarquías de dominancia, en la que los machos dominantes son los más grandes/viejos, pero dentro de machos de tamaño similar, es el tamaño relativo de la cabeza (empleada en las peleas) lo que determina su posición en la jerarquía (López et al. 2002; Aragón et al. 2004). El éxito reproductor de los machos depende de la búsqueda activa de hembras, es decir, de sus patrones de actividad, que, a su vez, depende de su condición física (Salvador et al. 2008).

Imagen 8. Macho (izquierda) y hembra (derecha) de lagartija carpetana (*Iberolacerta cyreni*) de la Sierra de Guadarrama (Madrid). Los machos presentan durante el celo una coloración dorsal verde azulada y ventral azulada, ocelos ventrales azules y producen secreciones céreas por los poros femorales (ver Imagen 9). Además, tienen cabezas proporcionalmente más grandes y un patrón más jaspeado que las hembras.

Imagen 9. Ocelos azules ventrales (a ambos lados) y secreciones céreas (amarillas) en los poros femorales de un macho de lagartija carpetana (*I. cyreni*) de la Sierra de Guadarrama durante el período de celo.

Los machos adultos más grandes presentan durante el celo una coloración verdoso-azulada (ver Imagen 8) y un número variable de ocelos azules en las escamas ventrales exteriores (ver Imagen 5 y 9). Estos ocelos reflejan en el ultravioleta y, como en el caso de otras especies de lacértidos, son utilizados como señales intrasexuales (Arribas 2001; Thorpe y Richard 2001; Molina-Borja et al. 1998; López et al. 2004). Además, los machos son capaces de discriminar los excrementos o las secreciones femorales (señales químicas; ver Imagen 9) dejadas en el sustrato por otros machos vecinos, posiblemente a nivel de individuo, y de utilizar la información que les proporcionan para decidir su uso del espacio o su posición jerárquica (López et al. 1998; Aragón et al. 2000, 2001a, b, c, d; Martín y López 2007). Las hembras también utilizan estas señales químicas para establecerse en territorios de machos de mayor calidad genética. Las hembras, sin embargo, no eligen las marcas químicas de los machos dominantes, sino de aquellos más viejos, en mejor condición corporal o de mayor calidad (más simétricos) o en mejor estado de salud (Martín y Salvador 1997b; Aragón et al. 2003, 2004; Martín y López 2000a,b, 2006a,b; López 2002; López y Martín 2002, 2003; López et al. 2002, 2003b, 2004). Además, aceptan las cópulas de estos machos con más facilidad que las de otros machos jóvenes o de menor calidad (López et al. 2003b).

Actividad

El periodo anual de actividad está generalmente limitado por habitar altas montañas. En la Sierra de Guadarrama está activa de finales de marzo o abril hasta primeros de octubre (Pérez-Mellado 1982). Su actividad diaria es bimodal, pero con un periodo vespertino más reducido que puede llegar a desaparecer en verano. Los machos están más activos que las hembras, y la actividad general es mayor durante la época reproductiva cuando tienen lugar los apareamientos (Martín y Salvador 1995, 1997b; Aragón et al. 2001b, 2004). Los machos dominantes están más activos, lo que les supone una pérdida mayor de peso, mientras que los machos subordinados están menos activos para disminuir los costes de agresión de individuos dominantes (Martín y López 2000b; Aragón et al. 2004). Las primeras hembras con huevos oviductales aparecen en junio-julio y existe una única puesta anual que tiene lugar en julio-agosto, siendo el tamaño medio de puesta entre 5,4 huevos en Gredos, 7,2 en Bejar y entre 5 y 8 en Guadarrama.

Depredación

Entre sus depredadores habituales se encuentran reptiles como la culebra lisa europea (*Coronella austriaca;* ver Imagen 2) y la víbora hocicuda (*Vipera latastei*). Entre las aves, probablemente sea depredada ocasionalmente por cuervos (*Corvus corax*), roqueros rojos (*Monticola saxatilis*) y alguna rapaz, como el Aguililla Calzada (*Hieraaetus pennatus*) (Martín y López 1990; Martín 1992; Salvador y Veiga 2003). Dentro de los mamíferos, los jóvenes pueden ser presa ocasional del topillo nival (*Chionomys nivalis*), que también ocasionalmente atacan adultos para hacerles desprenderse de la cola de la que se alimentan (López et al. 2003a), y probablemente sea capturada por comadrejas (*Mustela nivea*). Como táctica de escape utiliza carreras cortas para esconderse en refugios (generalmente grietas de rocas o bajo arbustos) y como último recurso defensivo presenta autotomía en la cola (Martín 1992, 2001, 2002a, b). Diversos estudios experimentales han demostrado que las decisiones de escape son dependientes de la temperatura corporal, distancia al refugio y costes del uso de refugios (costes fisiológicos debidos a temperaturas bajas) (Carrascal et al. 1992; Martín 2002; Martín y López 2000c, 2003) y el tiempo pasado en los refugios depende del balance entre el nivel de riesgo de depredación y los costes fisiológicos y de pérdida de oportunidades para alimentarse o reproducirse (Martín y López 1999, 2001, 2003, 2004; Martín et al. 2003a, b; Cooper et al. 2003; Polo et al. 2005). Además se han descrito variaciones interindividuales en cuanto a la propensión a arriesgarse en situaciones de peligro, que podrían ser interpretadas como diferentes "personalidades" (López et al. 2005).

La lagartija cantábrica

Aunque también se la considera una especie típicamente ligada a roquedos de alta montaña, en la Cordillera Cantábrica ocupa también rocas dentro de brezales y bosques caducifolios (Argüello y Salvador 1988; Moreira et al. 1998; Galán et al. 2007). Existen poblaciones a nivel del mar que aparecen ligadas a afloramientos rocosos y/o construcciones humanas en márgenes de ríos con ripisilva bien desarrollada, en tramos con pendientes del terreno acusadas, no encontrándose en puntos alejados del cauce del río (Galán 1999, 2007). Así, en Galicia y Asturias se encuentra tanto a baja altitud (50-90 m) como en bosques de montaña entre 650 y 1.550 m. (Braña 1984; Delibes y Salvador 1986; Galán y Fernández-Arias 1993; Galán 1999; Galán et al. 2007) llegando hasta casi los 2000 m en Serra da Estrela (Moreira et al. 1998). En la Cordillera Cantábrica se han estimado densidades de población medias de 52 ind./ha y máximas de 150 ind./ha (Delibes y Salvador, 1986), mientras que en Serra da Estrela varían entre 200-400 ind./ha en zonas bajas y 1.200 ind./ha en las zonas más altas (Moreira et al. 1998).

Organización social y selección sexual

También es una especie poligínica y los machos adultos defienden territorios de tamaño variable, sin embargo el solapamiento parece menor que en la lagartija carpetana (Galán, datos no publicados). En Serra de Estrela el tamaño del dominio vital de los machos parece depender de la densidad de población, oscilando entre 90 y 200 m^2, según disminuye la densidad. El tamaño del territorio de los machos disminuye al finalizar la época de los apareamientos, debido a una reducción de la actividad y movimientos. (Moreira et al. 1998). Los enfrentamientos agonísticos entre machos son frecuentes y los machos adultos (típicamente con coloración dorsal verdosa) guardan a las hembras y defienden territorios (Moreira 2002; Moreira et al. 1998).

Imagen 10. Macho (izquierda) y hembra (derecha) de lagartija cantábrica (*Iberolacerta monticola*) de A Capelada (Galicia). Los machos presentan durante el celo una coloración dorsal y ventral verde intensa y ocelos ventrales y axilares azules. Detrás del macho puede observarse los terrarios exteriores donde fueron mantenidos los individuos que participaron en los experimentos realizados para alguno de los capítulos de la presente Tesis Doctoral.

Los patrones de coloración están más desarrollados que en *I. cyreni*, lo cual sugiere que las señales visuales podrían ser más importantes en la comunicación sexual (Moreira 2002; Moreira et al. 1998; Galán 2008). Los machos adultos más viejos presentan durante el celo una intensa coloración verde y un número variable de ocelos azules en las escamas ventrales exteriores y en la zona axilar (Galán 2008; ver Imagen 3 y 10). Al igual que con la especie anterior, estos ocelos reflejan en el ultravioleta y podrían ser utilizados como señales intrasexuales (Arribas 2001; Thorpe and Richard 2001; Molina-Borja et al. 1998; López et al. 2004). Además, se sabe que los machos reconocen sus propias secreciones femorales de las de otros machos coespecíficos y que son capaces de percibir, a partir de estas señales químicas, el estatus de dominancia del otro macho para responder consecuentemente (Moreira et al 2006; Martín et al. 2007). Los machos más dominantes presentan una mayor concentracción de hexadecanol en sus secreciones femorales, que también está correlacionado con una mayor respuesta inmune (Martín et al. 2007).

Imagen 11. Aunque la mayor parte de los machos de lagartija cantábrica presentan coloraciones dorsales y ventrales verdes, de intensidad variable, algunos machos de las poblaciones gallegas presentan una coloración ventral, y sobre todo gular, azulada (Galán 2006).

Actividad

Aunque el periodo anual de actividad está generalmente limitado por habitar altas montañas, en zonas costeras de Galicia puede estar activa casi todo el año (Galán 1991). La actividad diaria es bimodal, pero con un periodo vespertino más reducido que puede llegar a desaparecer en verano. Los machos están más activos que las hembras, y la actividad general es mayor durante la época reproductiva cuando tienen lugar los apareamientos (Argüello y Salvador 1988). El periodo de apareamiento tiene lugar en Serra da Estrela entre mayo y junio, pero en Asturias y Galicia se adelanta a marzo y abril. Las primeras hembras con huevos oviductales aparecen en abril en las zonas costeras de Galicia. En Asturias, León, zonas costeras de Galicia y Serra de Estrela puede haber dos puestas, y no se descarta la existencia ocasional de una tercera (Rúa y Galán 2003).

Depredación

Entre sus depredadores habituales se encuentran reptiles como la culebra lisa europea (*Coronella austriaca*) (Barbadillo et al. 1997; Galán 1991) y la víbora hocicuda (*Vipera seoanei*). Entre las aves, probablemente sea depredada ocasionalmente por cuervos (*Corvus corax*), conejas (*Corvus corone*), urracas (*Pica pica*), alcaudones dorsirojos (*Lanius colurio*), y alguna rapaz, como cernícalos (*Falco tinnunculus*) o el busardo ratonero (*Buteo buteo*) (Martín y López 1990). Las tácticas de escape y estrategias antidepredadoras no han sido estudiadas, pero seguramente son similares a las de *I. cyreni* ■

Cuadro 2: Áreas de estudio

La caracterización del hábitat, observaciones comportamentales y captura de las lagartijas carpetanas se realizaron en diversos canchales de montaña de la Sierra de Guadarrama (Madrid), siempre por encima de los 1800 m. Este hábitat se caracteriza por la presencia de numerosos bloques de granito salpicados de matorrales de *Cytisus oromediterarneus* y *Juniperus comunnis* y rodeados de pinares (*Pinus sylvestris*).

Se escogió una población al nivel del mar, en A Serra da Capelada (San Andrés de Teixido, Galicia), para la captura, obsevación y caracterización del hábitat de la lagartija cantábrica. Las lagartijas se encontraban asociadas a grandes bloques de granito rodeados de abundante vegetación herbácea, matorrales de *Cytisus scoparius, Ulex europaeus* o *Rubus* sp.

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Objetivos

> *“The naturalist is a civilized hunter. He goes alone into the field or woodland and closes his mind to everything but that time and place, so that life around him presses in on all the senses and small details grow in significance. He begins the scanning search for which cognition was engineered. His mind becomes unfocused, it focuses on everything, no longer directed toward any ordinary task or social pleasantry.*
>
> Edward Osborne Wilson (Biophilia, 1984).

El objetivo general de esta Tesis es el de explorar las influencias que el ambiente puede tener sobre la evolución de las señales implicadas en los procesos de selección sexual. Entendemos “ambiente” como el escenario ecológico sobre el que actúa la selección natural, compuesto por factores tanto abióticos como bióticos. En particular, nos interesa saber qué factores pueden promover la divergencia entre poblaciones en cuanto al uso de distintos tipos de señales, y las consecuencias evolutivas de la triple interacción entre ambiente, comportamiento y señales, siempre en un contexto evolutivo.

Las lagartijas serranas (*Iberolacerta* sp.) ibéricas presentan unas condiciones muy adecuadas para acometer estos objetivos. En primer lugar, las distintas especies tienen distribuciones aisladas con características ecológicas diferentes y, en el caso de la lagartija carpetana (*I. cyreni*) numerosas poblaciones más o menos aisladas entre sí. En segundo lugar, presentan señales, tanto visuales, como químicas, y un sistema de selección sexual de gran complejidad. En tercer lugar, ya existe un conocimiento de determinados aspectos básicos y no tan básicos de su comportamiento adecuado para el planteamiento de determinadas cuestiones. Por último, las lagartijas son unos animales muy adecuados para el estudio del comportamiento y la evolución, debido a la facilidad con que pueden ser observadas, tanto en la naturaleza, como en el laboratorio; por su fuerte dependencia del medio; y por su gran diversidad de especies y variabilidad dentro de éstas.

En base a todo lo expuesto, nos planteamos los siguientes objetivos:

Capitulo 1

■ Es imposible interpretar las señales múltiples fuera de un contexto ambiental, dado que la percepción de cada señal es altamente dependiente de las características físicas del hábitat o de características sociales de la especie. Así, los animales podrían evolucionar para comunicarse a través de múltiples modalidades sensoriales en respuesta a ambientes fluctuantes o heterogéneos. Para comprobar esta hipótesis, se explora si el ambiente físico o social puede mediar la utilización de señales sexuales visuales o químicas en 15 poblaciones de lagartijas carpetanas (*Iberolacerta cyreni*) que presentan diferencias ambientales microgeográficas.

Capítulo 2

- La selección sexual favorece la utilización de señales conspicuas, mientras que la selección natural (a través del riesgo de depredación) favorece a los individuos más crípticos. Sin embargo, este compromiso podría verse alterado si los machos con una coloración más conspicua pudieran compensarla comportamentalmente, siendo más cautos con los depredadores. Para comprobar esta hipótesis, se explora si existe una regulación intraespecífica del comportamiento antidepredatorio dependiente de la conspicuidad visual y si las diferencias en conspicuidad de la coloración sexual que existen entre dos especies estrechamente relacionadas de lagartijas serranas (*Iberolacerta monticola* de Galicia e *I. cyreni* de Guadarrama) podrían mantenerse gracias a la compensación del comportamiento antidepredatorio.

Capítulo 3

- La conspicuidad de las señales químicas de los machos también puede atraer a los depredadores, pero los machos podrían compensarlo modificando su comportamiento antidepredador. Para comprobar esta hipótesis, se analiza si los machos de lagartija cantábrica (*Iberolacerta monticola*) que producen unas señales químicas más conspicuas para atraer a las hembras compensan comportamentalmente su exposición incrementada ante una culebra depredadora que usan esas señales químicas para localizar a sus presas.

Capítulo 4

- Diferentes sistemas de apareamiento pueden determinar los beneficios directos y/o indirectos que los machos proveen a sus parejas y, por tanto, sesgar las preferencias de las hembras por diferentes rasgos de las señales de los machos. Para comprobar esta hipótesis, se analiza si en las especie más territorial (*Iberolacerta monticola*), las hembras prefieren rasgos de las señales químicas de los machos relacionados con la dominancia, mientras que en la especie menos territorial (*Iberolacerta cyreni*) las hembras prefieren otros rasgos de las señales de los machos relacionadas también con su calidad genética heredable.

Capítulo 5

- La hipótesis de la especiación por selección sexual argumenta que la divergencia en las preferencias de las hembras por las señales de los machos locales puede causar un fuerte aislamiento reproductivo precópula. Para comprobar esta hipótesis, se explora en dos poblaciones aisladas de lagartija carpetana (*Iberolacerta cyreni*) las relaciones entre las características de las señales químicas de los machos, su calidad individual (medida como respuesta inmune) y las preferencias de las hembras, examinando si las variaciones geográficas en estas variables puede llevar a un aislamiento reproductivo.

Capítulo 1

INFLUENCIAS DEL AMBIENTE SOBRE LA SEÑALIZACIÓN MULTISENSORIAL EN DISTINTAS POBLACIONES DE LAGARTIJA SERRANA

Es imposible interpretar las señales múltiples fuera de un contexto ambiental, dado que la percepción de cada señal es altamente dependiente de las características físicas del hábitat o de características sociales de la especie. Así, los animales pueden evolucionar para comunicarse a través de múltiples modalidades sensoriales en respuesta a ambientes fluctuantes o heterogéneos. En este trabajo hemos explorado si el ambiente físico o social puede mediar la utilización de señales sexuales visuales o químicas en 15 poblaciones de lagartijas carpetanas (*Iberolacerta cyreni*) que presentan diferencias ambientales microgeográficas. Hemos encontrado que características del microhábitat tales como una mayor cobertura de canchales rocosos, suelo desnudo o mayor distancia a los refugios, pueden promover el uso de señales químicas, mientras que una mayor cobertura de plantas puede promover una señalización más visual. Tanto la competencia intrasexual (reflejada en el tamaño de la cabeza de los machos) como la territorialidad (reflejada por una heterogeneidad de hábitat) pueden promover una mayor inversión en la señalización tanto química como visual. Nuestros resultados muestran que las características físicas y sociales del ambiente de las lagartijas pueden jugar un papel importante en la evolución de la señalización multisensorial, y sugieren que el equilibrio entre cada modalidad sensorial en cada población puede depender de la interacción entre el ambiente social y las características del microhábitat ■

ENVIRONMENTAL INFLUENCES ON MULTI-MODAL SIGNALLING ACROSS IBERIAN ROCK LIZARD POPULATIONS

It is impossible to interpret multiple signalling outside the context of the environment, given that signal perception is highly dependent of habitat physical characteristics or social characteristics, and animals may evolve to communicate through multiple sensory modalities in response to fluctuating or heterogeneous environments. We explored whether physical or social environment may drive the use of visual or chemical sexual signals across 15 populations of *I. cyreni* lizards with microgeographic environmental differences. We found that microhabitat characteristics, such as higher cover of rocky outcrops, bare soil and farther distances from refuges, may promote chemical signalling, whereas higher cover of plants may promote visual signalling. Intrasexual competition (reflected by male head size) or territoriality (reflected by habitat heterogeneity) may promote both the development of visual and chemical signalling. Our results showed that physical and social characteristics of lizards' environment may play an important role in the evolution of multimodal signalling, and suggested that the equilibrium between each sensory modality in each population may depend on the interaction social environment and microhabitat characteristics.

Keywords: signal evolution, sexual selection, multiple signals, sensory modalities, chemical signals, visual signals, lizards

It has been known for years that multiple sensory systems (or modalities) are im-portant for communication (Darwin 1872; Tinbergen 1959). However, the relationships among different sensory channels have been little studied but until recently (Partan and Marler 2005). Many animals produce and respond to displays made up of multiple components (for review see Rowe 1999; Candolin 2003). These signals have been described as being 'multicomponent' (Hölldobler 1995; Johnstone 1995; Kodric-Brown and Nicoletto 2001), or 'multimodal' where components occur in more than one sensory system (Guilford and Dawkins 1991; Rowe and Guilford 1999; Elias et al. 2005; Partan and Marler 2005).

Most theoretical interest has focused upon explaining multiple sexual displays, exploring the conditions under which multiple handicaps or Fisherian traits might evolve (reviewed in Candolin 2003). However, focusing purely on the honesty of signals, omits other selection pressures over the design features of signals that enhance effective communication (Alberts 1992; Endler and Basolo 1998; Endler et al. 2005; Hebets and Papaj 2005). Many studies show that signal differences among different species or populations are consistent with the idea that selection has favoured more effective stimulation of receivers that differs under differing environments (e.g., Endler and Houde 1995; Endler and Théry 1996; Boughman 2001; Leal and Fleishman 2004). Most of these studies rely on differences in exclusively one sensory system, but accurate and efficient mate recognition and assessment may also require that individuals of different species or populations use the sensory system best suited for their mating environments. However, the role of the environment in the evolution of sexual signals perceived under different sensory modalities remains, to our knowledge, almost unexplored.

The goal of this study was to explore whether differences in physical or social environment may drive the use of sexual signals based on different sensory systems in a lizard species with multisensory (visual and chemical) signalling. Studies using comparative methods have investigated the evolutionary histories of visual (Macedonia 2001; Ord et al. 2001; 2002; Stuart-Fox and Ord 2004; Ord and Stuart-Fox 2006; Stuart-Fox et al. 2007) and chemical signalling (Kratochvil and Frynta 2002; Pincheira-Donoso et al. 2008) in various groups of lizard species. However, no previous studies have explored the evolutionary interaction of both traits in relation to physical or social environment. Moreover, most of studies have involved comparisons across species. A within-species comparison may provide a better test of the effect of habitat as it provides greater control of other factors that can influence signalling, such as phylogenetic history (Ryan and Brenowitz 1985) or body size, which may affect the size, type, and transmission distance of visual cues (Bradbury and Vehrencamp 1998; Podos 2001). Thus we examined, across 15 populations of Carpetane rock lizards (*Iberolacerta cyreni*), the relationships between the use of chemical and visual signalling, the most prominent lizard sensory systems, and different physical and social environmental factors.

The Carpetane rock lizard, *I. cyreni* (formerly *Lacerta monticola cyreni*) is a lacertid lizard found at mountains in Central Spain, occupying sparsely vegetated rocky areas at high elevations (above 1700 m) (Martín 2005). For a variety of reasons, *I. cyreni* is an excellent candidate to test environment effects on sensory systems. First, mountain topography and historical climatic changes have led to different isolated populations, which are geographically very close, but isolated by the presence at lower altitudes of surrounding pine forests where this lizard does not occur (Martín 2005). This fragmented population system is especially adequate to examine the topic of the present study because provides geographically isolated lizard populations which presumably experience varying environmental conditions (e.g., with different sun orientations, vegetation types or topographical features). Second, *I. cyreni* use both visual and chemical signals in a mating context. As in many lacertid lizards, male *I. cyreni* show conspicuous blue spots on lateral-ventral scales which reflect ultraviolet (UV) light (Arribas 2001; Thorpe and Richard 2001) and are used in male contests (Molina-Borja et al. 1998; López et al. 2004). Chemical signalling is also well-developed in this species, where scent marks from femoral pores of males convey information about social status (Aragón et al. 2001; Martín and López 2007, 2008; Martín et al. 2007) or about traits used by females in mate choice (Martín and López 2000, 2006a,b; López et al. 2002, 2003). The femoral pores could be considered as a secondary sexual trait subjected to sexual selection (Martín and López 2000) and the number of femoral pores may vary considerably among species (e.g., only two or three pores occur on each leg in *Sceloporus horridus*, whereas the others *Sceloporus* species have several times that many). An increase in the number of femoral pores implies an increase in the amount of holocrine femoral glands which secrete pheromone components (Escobar et al. 2003; Martín and López 2006a). Thus, we used population average number of blue spots as an indirect measure of the intensity of use of visual signalling, and population average number of femoral pores as an indirect measure of the intensity of use of chemical signalling.

To examine environmental influences on sexual signalling we characterized the microhabitats available and selected by lizards in each population. The relative conspicuousness of a signal can be strongly affected by how well it transmits through the local environment, that is, how well it can be seen or smelled from a distance. For instance, habitat openness or vegetation cover affects light conditions, which may exert strong selection on visual signals such as colour patches or ornaments to maximize conspicuousness (Marchetti 1993; Endler, 1992, 1993; Endler and Thery 1996; Leal and Fleishman 2004). Predators may also exploit signals to localize prey (Endler 1978, 1980; Zuk and Kolluru 1998), and habitat openness can in turn determine the vulnerability of signallers to visual predators. However, other environment characteristics, such as humidity, could drive the use of chemical signalling (Alberts 1992). For instance, hydrophobicity makes the waxy pore secretions difficult to detect by olfactory organs and humidity increases the degradation of femoral pore secretions in substrate scent marks, reducing their durability. Therefore, changes in physical habitat characteristics could drive the use of signals from one sensory system to another.

Social environment may also confer different advantages associated to each sensory modality. Spacing patterns may influence the distance over which a signal must function and social factors, as intrasexual competition, also influence signalling (Andersson 1994; Blumstein and Armitage 1997; Ord et al. 2001; 2002). In lizards, "space distribution" is mediated by refuge availability (Van Damme et al. 1989; Lemos-Espinal and Ballinger 1995; Martín and Salvador 1997) and thermoregulation requirements (Huey 1982; Sorci et al. 1996; Bashey and Dunham 1997). Thus, the distribution and abundance of refuge and thermoregulatory appropriate microhabitats should predict male and female spatial distribution in space and time and may have promoted different social scenarios. Encounters between males during the breeding season often escalate into fights with vigorous biting (Martín and López 2007; Martín et al. 2007). Male head size clearly indicate a dimorphism in bite performance (Herrel et al. 1999), suggesting that intrasexual competition may be a driving force for the head sexual dimorphism (Olsson 1992; Stamps et al. 1997; Molina-Borja et al. 1998; Gvozdík and Van-Damme 2002; Baird et al. 2003). Thus, we also used relative head size of males as indirect measure of among population social differences (Stuart-Fox and Ord 2004).

In short, we examined the relationship between two of the most prominent display sexual traits of lizards, chemical and visual signals, and physical or social environment characteristics across 15 isolated populations of *I. cyreni* lizards with microgeographic environmental differences.

Methods

Study area

We did field work during spring-summer 2003 at 15 different localities covering the geographic distribution of Iberian rock lizards in the Guadarrama Mountains (Central Spain) (Martín 2005) (Table 1). We searched for lizards by walking between 07:00 and 14:00h (GMT) with consistent sampling effort in all microhabitats and study localities. We captured adult male and female lizards by noosing. The body size distribution of lizards in the samples reflected the adult size distributions typical for these populations.

Lizard characteristics

We weighed all captured lizards with a Pesola spring scale (to the nearest 0.1 g), and measured their snout-vent-length (SVL) with a ruler to the nearest 1 mm, and their head length, width and depth with a digital calliper to the nearest 0.05 mm. We used a principal component analysis (PCA) to reduce the three head measures (length, width and depth) and SVL (all log transformed) to a single component representing relative head size (hereafter 'head size'). We considered that higher levels of intramale competition would result in relatively greater head sizes of males, and consequently in a higher degree of sexual dimorphism.

We also counted in all captured males the number of femoral pores on the right and left hind legs with the aid of a magnifying glass, and the number of ventral blue spots on the right and left sides of the belly (2–4 counts performed per animal in both cases). Numbers of blue spots and femoral pores did not change in the same individual within the same reproductive season (unpublished data).

Microhabitat characteristics

We also recorded microhabitat data at the point where each captured male lizard was first sighted. Four 1 m transects were laid out radiating from this point along the four cardinal directions, and records made at 5, 10, 15, 25, 75 and 100 cm. We noted the presence at substrate level of grass, leaf litter, bare sandy soil, small rocks (< 25 cm), medium rocks (25-100 cm), large rocks (100-200 cm), or rocky outcrops and cliff walls (> 200 cm). Plant contacts at 5, 10, 25 and 50 cm height were also noted, using a calibrated stick, for grasses (e.g., *Festuca indigesta*, *Koeleria caudata*), ferns (e.g., *Cryptogramma crispa*, *Asplenium trichomanes*, *Dryopteris oreades*) and small herbaceous semi-perennial plants (<50 cm height; e.g., *Senecio pyrenaicus*, *Digitalis purpurea*, *Doronicum carpetanum*), large woody bushes (*Cytisus oromediterarneus* and *Juniperus comunnis*) or trees (*Pinus sylvestris*). We also noted whether the sample point was in a sunny or shaded location, the presence of canopy tree cover above each point, and the distance to the

Table 1. Male lizard average characteristics (mean ± SE) and altitude of lizards' populations.

Population	SVL (cm)	Head size (PC scores)	Femoral pores	Blue spots	Altitude (m)
Casa Derruida	75.4 ± 1.9	0.74 ± 0.3	19.1 ± 0.4	6.1 ± 0.8	1970
Cueva	72.7 ± 1.1	0.52 ± 0.2	18.6 ± 0.2	5.4 ± 0.8	1935
Siete Picos	77.5 ± 1.7	0.27 ± 0.4	18.7 ± 0.5	8.7 ± 1.0	2080
Trigo	75.5 ± 1.2	0.75 ± 0.2	18.0 ± 0.4	8. 7 ± 0.7	1965
Minguete	77.6 ± 1.4	0.60 ± 0.2	18.6 ± 0.4	7.4 ± 0.8	2010
Bola	73.8 ± 1.3	-0.03 ± 0.2	17.9 ± 0.3	5.6 ± 0.9	1940
Cabrillas	73.9 ± 2.1	-0.29 ± 0.4	17.9 ± 0.3	5.6 ± 1.1	1960
Dos Castillas	75.5 ± 2.6	-0.49 ± 0.5	17.8 ± 0.4	6.4 ± 1.1	2150
Antenas	77.3 ± 1.4	0.13 ± 0.2	17.8 ± 0.3	6.3 ± 1.2	2250
Cancho Negro	80.0 ± 1.3	0.57 ± 0.2	18.2 ± 0.3	7.7 ± 0.8	2170
Valdemartín	79.3 ± 1.1	0.46 ± 0.2	18.2 ± 0.4	6.0 ± 0.9	2270
Topillo 1	71.5 ± 0.7	-0.07 ± 0.1	18.0 ± 0.3	6.6 ± 1.2	1880
Topillo 2	72.1 ± 0.8	-0.19 ± 0.2	17.7 ± 0.4	5.4 ± 1.0	1887
Valdesquí	76.3 ± 1.5	-0.01 ± 0.2	18.5 ± 0.4	3.7 ± 0.7	1960
La Peñota	82.7 ± 1.1	0.54 ± 0.2	18.5 ± 0.3	7.4 ± 1.0	1940

nearest potential refuge (rock crevice or protective vegetation cover). This procedure provided 24 sample points per lizard location and allowed us to calculate the percent cover values of each habitat variable (i.e., % contacts with each substratum, vegetation type and sunny spots, and the mean distance to the nearest refuge; for a similar sampling methodology see Martín and Salvador, 1997). To estimate the availability of microhabitats in a large area surrounding that actually used by lizards, we used a similar procedure to record the same variables as described above at 2, 3 and 4 m. along the four cardinal directions from each sample point.

We used a principal component analysis (PCA) to reduce the microhabitat variables to a smaller number of independent components. Original data (number of contacts) were normalized by means of square-root transformation. Because of the large number of "zero" observations for many variables, some transformed data were not normally distributed, but otherwise met the assumptions of a PCA. Deviations from normality do not necessarily bias the analysis, as long as the distributions are reasonably unskewed (Legendre and Legendre 1998). Thus, we considered that our transformations reduced the skew sufficiently to minimize the risk of bias in the analysis. Only principal components (PCs) with eigenvalues that explained more than 5% of the total variation were retained for further analysis. Thus, the PCA for microhabitats available and those used by lizards produced six components that together accounted for the 67.43 % of the variance (Table 2). Then we calculated the mean values of each PC for microhabitats available and used for each population to use them as predictor variables in posterior analyses. Finally, altitude captures much of the variation in factors such as temperature, humidity, and wind intensity (Escobar et al. 2001; Pincheira-Donoso et al. 2008) which may influence chemical signalling (Alberts 1992). Thus, we noted the altitudinal midpoint for each population to be used as an additional predictor variable.

We also calculated for each population the average difference between PC scores describing microhabitat available and used by lizards. We considered that this measure may affect the intensity of intramale competition for occupying selected microhabitats, such that competition would be greater in populations where microhabitats used differed more from available ones (i.e., where preferred microhabitats represented a smaller proportion of the total available area).

Relationships between habitat and lizard characteristics

We used general linear regression models (GRM) to estimate relationships between mean numbers of blue spots or femoral pores of males and environmental variables (microhabitat PCs, altitude and head size). Each type of signal (numbers of blue spots or femoral pores) was analysed separately as the dependent variable, using as potential predictors the physical environmental variables (i.e., all the PCs extracted from microhabitat, altitude and SVL) or the social environmental variables (head size and the magnitude of the differences between microhabitat available and used for each PC).

All variables were log transformed prior to analyses. Data normality was verified by Shapiro-Wilk's tests, and tests of homogeneity of variances (Leveane's test) showed that variances were not significantly heterogeneous after transformation. We used a "best-subsets" approach in conjunction with stepwise methods (Neter et al. 1985). We chose the most parsimonious model as having the lowest Akaïke information criterion (AIC), and we checked its coincidence with the equivalent stepwise solution. Residuals from the final models were normally distributed (Grafen and Hails 2002). All statistical analyses were performed with the software Statistica version 6.0. All tests were two-tailed and the level of significance was 0.05.

Results

Interpopulation variation in lizard characteristics

There were significant differences between males from different populations in snout-to-vent length, SVL (one-way ANOVA, $F_{14,200} = 5.42$, $P < 0.0001$), and in the number of blue spots ($F_{14,200} = 2.19$, $P = 0.009$), but not in the number of femoral pores ($F_{14,200} = 1.17$, $P = 0.29$) (Table 1). There were weak but positive and significant relationships between SVL of male lizards and the number of femoral pores (Pearson's correlation, $r = 0.20$, $F_{1,213} = 9.18$, $P = 0.02$) and blue spots ($r = 0.22$, $F_{1,213} = 11.53$, $P = 0.0008$). The number of femoral pores was no significantly related to the number of blue spots ($r = 0.11$, $F_{1,213} = 2.84$, $P = 0.09$).

Relative head size was significantly larger in males than in females (two-way ANOVA, sex effect: $F_{1,291} = 48.00$, $P < 0.0001$) and varied significantly between populations (population effect: $F_{1,14} = 2.34$, $P = 0.004$). The interaction between population and sex approached significance ($F_{1,14} = 1.68$, $P = 0.057$) suggesting that the magnitude of the differences in head size between males and females (i.e, sexual dimorphism) tended to differ between populations.

Interpopulation variation in habitat characteristics

There were significant differences in relation to all PCs describing habitat characteristics among populations (GLM, Wilks' $\chi^2 = 0.19$, $F_{84,2219} = 9.20$, $P < 0.0001$) and between types of microhabitat points (available *vs.* used by lizards; Wilks' $\chi^2 = 0.12$, $F_{6,397} = 482.31$, $P < 0.0001$). The interaction between population and type of point was significant (Wilks' $\chi^2 = 0.24$, $F_{84,2219} = 9.20$, $P < 0.0001$) showing that the magnitude of the differences between available and used habitats differed among populations. The general model showed significant overall differences between microhabitats available and used by lizards for all PCs (adjusted $R^2 > 0.11$, $F_{29,402} > 2.85$, $P < 0.0001$ in all cases) but for PC-4 (adjusted $R^2 = 0.003$, $F_{29,402} = 1.04$, $P = 0.40$). Thus, microhabitats used by lizards had significantly lower cover of *Cytisus* bushes and leaf litter (PC-1), *Juniperus* bushes (PC-2), plants (PC-3), grasses, small and medium rocks and sunny sites (PC-5), rocky outcrops and bare soil (PC-6) and are closer to refuges (PC-6) than microhabitats available.

Relationships between lizard and habitat characteristics

With respect to the physical environment, considering the average values for each population, the SVL of male lizards was not significantly related to any of the PCs describing habitats used by lizards, which were not included in the final stepwise GRM model, but SVL was significantly and positive related to population altitude midpoint (stepwise best subsets GRM, adjusted $R^2 = 0.34$, $\beta = 0.64$, $F_{2,12} = 4.67$, $P = 0.03$). Thus, habitat characteristics did not affect body length (SVL) of male lizards, but lizards were larger at higher altitude.

Table 2. Principal component analyses for microhabitats available and used by lizards. Correlations marked in bold are significant at $P < 0.0001$.

	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6
Ground level:						
Small rocks (< 25 cm)	0.14	-0.01	-0.06	-0.12	0.63	**-0.19**
Medium rocks (25-100 cm)	-0.09	0.29	0.06	-0.03	**0.54**	**-0.02**
Large rocks (100-200 cm)	**-0.20**	0.16	0.02	0.03	**0.20**	**0.39**
Rocky outcrops (> 200 cm)	0.08	-0.09	-0.09	0.01	-0.14	**0.76**
Bare sandy soil	0.15	0.20	0.15	0.04	0.04	**0.57**
Grasses	0.23	0.03	-0.01	0.04	**0.76**	0.16
Leaf litter	**0.64**	**0.48**	0.22	0.01	0.08	0.07
Plant contacts:						
Grass (5 cm)	0.26	-0.03	0.01	0.04	**0.79**	0.20
Grass (10 cm)	0.21	-0.01	0.09	0.04	**0.36**	0.45
Grass (25 cm)	0.08	0.05	**0.19**	0.03	0.07	0.35
Grass (50 cm)	-0.02	0.06	0.01	-0.99	0.02	0.02
Ferns (10 cm)	-0.02	0.06	0.01	**-0.99**	0.02	0.02
Ferns (25 cm)	-0.02	0.06	0.01	**-0.99**	0.02	0.02
Plants (5 cm)	0.07	-0.02	**0.95**	0.01	-0.01	0.08
Plants (10 cm)	0.03	-0.03	**0.97**	0.01	0.01	0.09
Plants (25 cm)	0.03	-0.03	**0.97**	0.01	0.01	0.09
Cytisus (5 cm)	**0.86**	0.06	0.19	0.02	0.12	0.12
Cytisus (10 cm)	**0.87**	0.09	0.19	0.03	0.15	0.13
Cytisus (25 cm)	**0.89**	0.07	0.09	0.03	0.14	0.13
Cytisus (50 cm)	**0.88**	0.05	0.01	0.01	0.11	0.12
Cytisus (75 cm)	**0.68**	0.03	-0.13	0.01	0.06	-0.02
Cytisus (100 cm)	**0.58**	-0.10	-0.12	-0.02	0.06	-0.05
Juniperus (5 cm)	0.01	**0.93**	-0.01	-0.05	0.14	0.03
Juniperus (10 cm)	0.01	**0.94**	-0.01	-0.04	0.17	0.04
Juniperus (25 cm)	0.02	**0.95**	-0.02	-0.02	0.15	0.02
Juniperus (50 cm)	0.04	**0.86**	-0.02	-0.12	0.01	0.04
Juniperus (75 cm)	0.19	**0.40**	-0.06	0.04	-0.15	0.15
Ditance to refuge	0.07	**-0.02**	-0.06	0.03	0.50	**0.76**
Sunny location	**0.10**	**0.18**	0.02	-0.02	**0.81**	0.40
Canopy tree cover	0.01	0.01	0.02	-0.10	**0.01**	0.21
Eigenvalue	6.31	3.88	2.94	2.83	2.72	1.56
% Variance	21.02	12.93	9.79	9.43	9.07	5.19

The average number of femoral pores of male lizards was positively and significantly related with PC-6 describing microhabitats used by lizards (stepwise best subsets GRM, adjusted $R^2 = 0.35$, $\beta = 0.63$, $F_{1,13} = 8.46$, $P = 0.012$) (Fig. 1a). Thus, male lizards from populations with microhabitats that had higher cover of rocky outcrops and bare soil, and that are far from refuges, had an average larger number of femoral pores, independently of SVL variations, which was not included in the final model. We obtained similar results when considering the PCs describing available microhabitats.

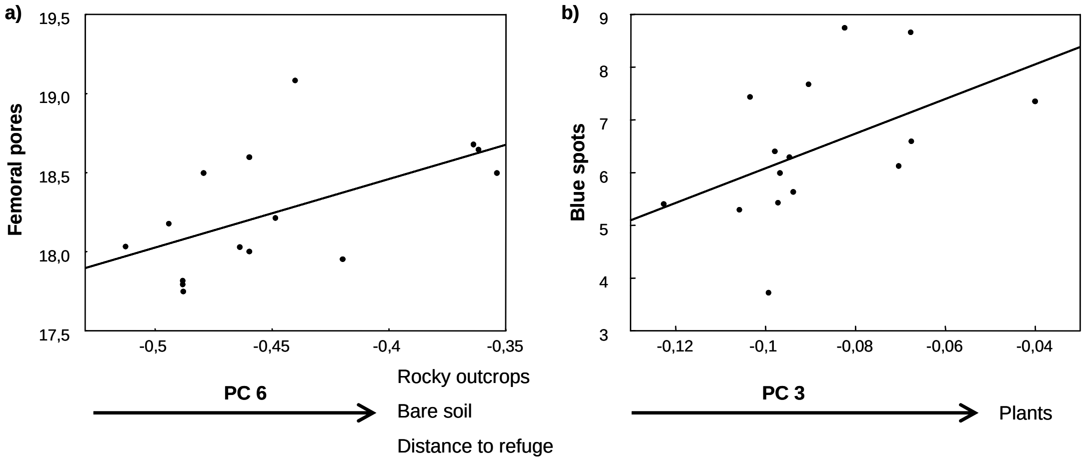

Fig. 1. Relationship between the average number of a) femoral pores and b) blue spots of males in each population and PCs for characteristics of microhabitat used by lizards.

The average number of blue spots of male lizards was positively and significantly related to PC-3 describing microhabitats used by lizards (stepwise best subsets GRM, adjusted $R^2 = 0.26$, $\beta = 0.56$, $F_{1,13} = 5.84$, $P = 0.031$) (Fig. 1b). Thus, male lizards from populations with microhabitats with higher cover of plants had an average larger number of blue spots, independently of SVL variations, which was not included in the final model. We obtained similar results when considering the PCs describing available microhabitats.

With respect to the social environment, the average number of femoral pores of male lizards was positively and significantly related with average head size and with the magnitude of the differences between microhabitat available and used explained by PC-6 (stepwise best subsets GRM, model: adjusted $R^2 = 0.60$, $F_{2,12} = 11.41$, $P = 0.001$; head size: $\beta = 0.48$, $t = 2.44$, $P = 0.03$; PC-6: $\beta = 0.44$, $t = 2.21$, $P = 0.04$) (Fig. 2). Male lizards from populations where conspecific males had average relatively greater head size and where microhabitats available and used differed more in the cover of rocky outcrops and bare soil and in distances to refuges, had an average larger number of femoral pores.

The average number of blue spots of male lizards was positively and significantly related with average head size and with the magnitude of the differences between microhabitat available and used explained by PC-3 (stepwise best subsets GRM, model: adjusted $R^2 = 0.48$, $F_{2,12} = 7.40$, $P = 0.008$; head size: $\beta = 0.50$, $t = 2.61$, $P = 0.02$; PC-3: $\beta = 0.51$, $t = 2.66$, $P = 0.02$) (Fig. 3). Thus, male lizards from populations where conspecific males had average relatively greater head size and where microhabitats available and used differed more in plant cover had an average larger number of blue spots.

Average head size was significantly and positively correlated with the difference between microhabitats available and used described by the PC-6 (GRM, adjusted $R^2 = 0.21$, $\beta = 0.52$, $F_{1,13} = 4.93$, $P = 0.04$). Thus, populations where microhabitats available and used differed more in the cover of rocky outcrops and bare soil and in distances to refuges had males with greater head size (Fig 4).

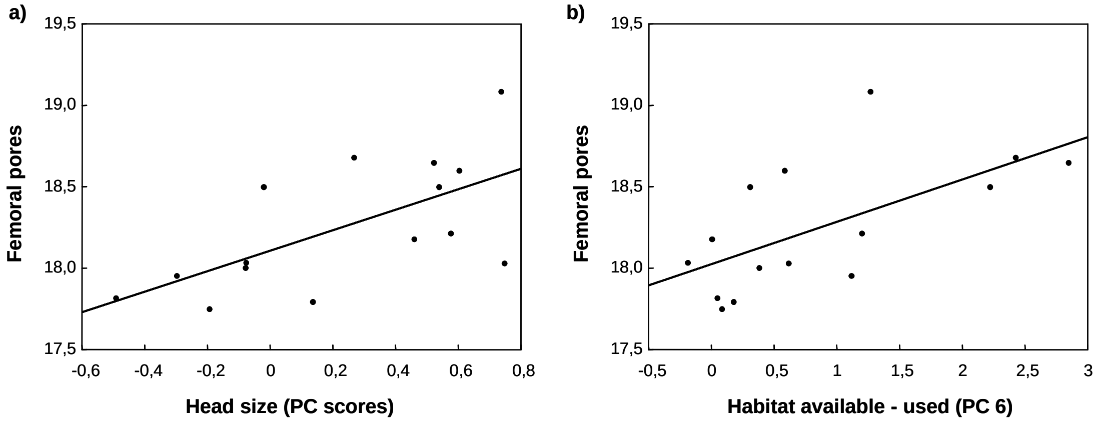

Fig. 2. Relationship between the average number of femoral pores of males in each population and a) average relative head size of conspecific males or b) differences between characteristics of microhabitats available and used by lizards (related to cover of rocky outcrops and bare soil; PC 6).

Discussion

Our results showed that different physical and social environmental characteristics may drive the divergence of chemical or visual signalling among *I. cyreni* lizard populations. We found general interpopulation differences in habitat characteristics (substrate and vegetation cover) that reflected differences in physical characteristics (e.g., temperature and humidity, visibility) which may influence the efficiency of both chemical and visual signalling (Endler and Houde 1995; Endler and Thery 1996; Alberts 1992; Leal and Fleishman 2004). Moreover, we also found interpopulation differences in the interaction between available and used habitats and in male head size (related with male aggressiveness; Herrel et al. 1999), suggesting differences in social characteristics (intensity of male intrasexual competition), which may also influence signal evolution (Andersson 1994; Blumstein and Armitage 1997; Ord et al. 2001). Recent studies have compared the relationship between two sensory types (visual and acoustic signals) elaborations across bird species (Badyaev et al. 2002; Price et al. 2006). Animals may evolve to communicate through multiple sensory modalities in response to a fluctuating or heterogeneous environment (reviewed in Candolin 2003; Partan and Marler 2005), and different populations or species may experience varying ecological conditions that may influence the use of each sensory modality. Two primary hypotheses have been proposed to explain the use of multiple traits in mate choice: the “redundant” (or “back-up”) signal hypothesis and the “multiple messages” hypothesis (Møller and Pomiankowski 1993; Johnstone 1996; Candolin 2003; Hebets and Papaj 2005; Partan and Marler 2005). However, it is impossible to interpret multiple signalling outside the context of the environment, given that signal perception is highly dependent of habitat physical characteristics (Endler 1992; Endler and Basolo 1998; Bradbury and Vehrencamp 1998; Chiao et al. 2000) or social characteristics (Andersson 1994; Blumstein and Armitage 1997; Ord et al. 2001).

Influence of physical characteristics

Our results showed that different physical factors of the environment may drive each sensory modality, and that population equilibrium between both sensory modes should be reached depending on habitat characteristics. Thus, we found that microhabitat characteristics, such as the higher cover of rocky outcrops, bare soil and farther distances from refuges, may promote chemical signalling in male *I. cyreni* lizards, whereas higher cover of plants may promote visual signalling.

Changes in environmental conditions (e.g., microhabitat structure or predation risk) that lead to change in signal detectability, or differences in the cost of some traits, can strongly influence both preference for traits and trait elaboration (Darwin 1871; Endler 1992; Leal and Fleishman 2004). With respect to visual signals, the habitat in which natural and sexual selection occurs is divided into microhabitats that differ in physical properties that influence colour perception, such as light intensity, light spectrum, and/or substrate colour and pattern (Endler 1993; Gomez and Théry 2004). To be effective, colour signals should be conspicuous to intended receivers, given the visual background and prevailing light conditions (Endler 1992; Fleishman and Persons 2001; Macedonia 2001; Stuart-Fox et al. 2007). Habitat openness or vegetation cover affects light conditions, which may exert strong selection on visual signals such as colour patches or ornaments to maximize conspicuousness (Marchetti 1993; Endler and Théry 1996; Zahavi and Zahavi 1997; Leal and Fleishman 2004). We found that male lizards from populations with more plant cover had more blue spots. These blue spots reflect ultraviolet (UV) light (Arribas 2001; Thorpe and Richard 2001). The conspicuousness of UV signals is particularly highlighted in dark environments, such as inside refuges or under vegetation cover, because the radiance from the background is considerably lower for the UV light than in open areas (Endler 1993; Hunt et al. 2003; Avilés et al. 2006). Therefore, in habitats with more plant cover (i.e., shadier environment) blue spots might be more efficient and advantageous than in open habitats. Predators may also exploit signals to localize prey (Endler 1978, 1980; Ryan 1987), and bright colours, habitat openness or refuge availability can in turn determine the vulnerability of signallers to visual predators (Stuart-Fox and Ord 2004; Cabido et al. in press). Blue spots may increase predation risk because diurnal predators use UV wavelengths as a hunting cue (Viitala et al. 1995, Honkavaara et al. 2002; Probst et al. 2002). Thus, a lower availability of refuges or farther average distances to refuges may also influence the advantages of using blue spots, limiting the use of conspicuous visual signals and promoting the use of alternative signals such as chemical ones, as we have found. Therefore, if predation limits the use of visual signals, chemical signalling may replace visual signalling as the target of sexual selection (Darwin 1871; Shutler and Weatherhead 1990), thus explaining the positive relationship between refuge availability and number of femoral pores.

Lizards inhabiting more mesic tropical habitats vs. those in xeric habitats might use chemical signals differently because of possible differences in persistence times of the chemical signal (Gravelle and Simon 1980). A higher cover of plants offers a higher protection from predators (Martín and López 1995), but it is also related with higher humidity levels, which limits the duration of chemical signals (Alberts 1992). Therefore, in those populations where humidity is high and predation pressure is low, visual elaboration may replace chemical signals as the target of sexual selection.

Therefore, our results showed that microhabitat, through its influence both on properties of the signalling environment and predation pressure, can thus play an important role in the evolution of multimodal signalling in lizards, and suggest that the equilibrium between each sensory modality may depend on the interaction of

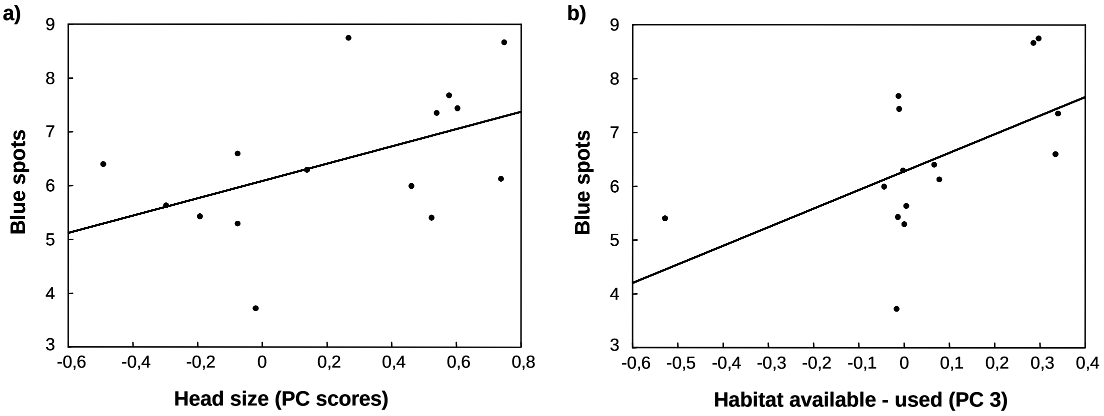

Fig. 3. Relationship between the average number of blue spots of males in each population and a) average relative head size of conspecific males or b) differences between characteristics of microhabitats available and used by lizards (related to plant cover; PC 3).

the different habitat characteristics of each population. In this sense, Hews and Benard (2001) found a negative association between visual and chemical signalling in phrynosomatid lizards. Although their results are limited because they involve only a single species-pair comparison, we did not find a significantly relationship between the population average number of femoral pores and the number of blue spots. This could be due to different relationships (positive or negative) depending on the weight of the different factors in each population. For instance, social organization may promote both chemical and visual signalling (see below), that is, a positive relationship between femoral pore and blue spots number, whereas microhabitat may promote only one of them or favour a negative relationship between both. Thus, the additive and interacting whole effects of all environmental factors may hide a simple interaction between sensory modalities.

Several phylogenetically controlled comparative analyses have also found that habitat characteristics influence visual signal repertory in iguanian lizards (Macedonia 2001; Ord et al. 2001, 2002), agamid lizards (Stuart-Fox and Ord 2004) and chameleons (Stuart-Fox et al. 2007). However, Pincheira-Donoso et al. (2008) in a phylogenetically controlled comparative analysis found that environmental conditions failed to predict chemical signalling (number of precloacal pores) across several species of lizards of the *Liolaemus* genus, being the phylogenetic relationships a strong predictor of the number of precloacal pores. However, the comparison across species may suppose a lack of control of other factors different of habitat characteristics that can also influence signalling, such as body size (Bradbury and Vehrencamp 1998; Podos 2001) or variation in social organization (present study, see below; Ord et al. 2001; Ord and Stuart-Fox 2006). On the contrary, our study compares different closely related populations which have been recently isolated (*I. cyreni* only occurs above 1700 m in the top of Guadarrama mountains, alike “mountain islands”) that only differ in environmental conditions (see also Leal and Fleishman 2004). In addition, Pincheira-Donoso et al. (2008) used the latitude and altitude of each species distribution as indirect measures of temperature, wind and humidity. The lack of relationships between number of femoral pores and habitat could be due to a habitat classification based in inaccurate measures, or factors that do not directly affect the efficiency of chemical signals. Our study involving multiple quantitative measures of how habitat differs physically are potentially more statistically robust as it tests for consistent trends within each habitat.

Influence of social characteristics

Our study shows that populations where microhabitats available and used differed more in the cover of rocky outcrops and bare soil and in distances to refuges, that is with higher habitat heterogeneity related with territoriality (Emlen and Oring 1977; M'Closkey et al. 1990a,b; Perry and Garland 2002), had males with greater head size which is related with higher levels of male competition (Herrel et al. 1999) and intrasexual selection (Gvozdík and Van-Damme 2002; Stuart-Fox and Ord 2004). Male lizards from these populations also had an average larger number of femoral pores. In contrast, male lizards from populations with average relatively greater male head size, but where microhabitats available and used differed more in plant cover, had an average larger number of blue spots. Several comparative (Ord et al. 2001; Stuart-Fox and Ord 2004) or experimental studies (Baird et al. 1997) showed the influence of intrasexual competition and social organization in the evolution of visual signalling, suggesting that signal complexity has evolved in order to improve opponent assessment under conditions of high male-male competition. Kratochvil and Frynta (2002) also showed, in a study of the relationships between male territorial behaviour and presence /absence of precloacal glands in eublepharid geckos, that species lacking these structures are less territorial. Our results suggest that intrasexual competition (reflected by male head size) or territoriality (reflected by habitat heterogeneity) may promote both the development of visual and chemical signalling. However, habitat heterogeneity due to differences in rocky cover drives chemical signalling, whereas plant cover drives visual signalling. Social systems and spacing patterns influence the distance over which a signal must function (Andersson 1994; Blumstein and Armitage 1997; Ord et al. 2001, 2002) and olfactory cues have traditionally been classified as long-distance signals (Bradbury and Vehrencamp 1998). In populations where male have to compete for rocky outcrops (which are preferably selected by *I. cyreni*; Martin and Salvador 1997) chemical cues may function to alert the receiver of the presence of the visual cues, increasing the probability of their detection and recognition (Rowe and Guilford 1999; Rowe 1999; López and Martín 2001). Moreover, chemical signal durability may be longer on rocky substrates (Gravelle and Simon 1980) that with the long-distance detection of olfactory signals may combine to decrease the costs incurred by a female during the mate selection process, and increase her probability of finding a mate (Real 1990; Jumper and Baird 1991). On the contrary, in populations where habitat heterogeneity is due to plant cover (which is avoided by *I. cyreni*; Martín and Salvador 1997), chemical signals may have less duration and visual signals may be more advantageous. These results suggest that,

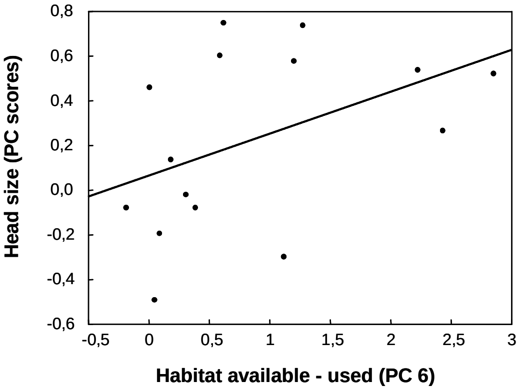

Fig. 4. Relationship between average head size and the difference between microhabitats available and used described by the PC-6.

although sexual competition promote signal complexity (e.g., a higher number of femoral pores or blue spots), habitat characteristics drives the efficiency and favours the use of each sensory mode.

In summary, our results showed that both physical and social characteristics of lizards' environment may play an important role in the evolution of multimodal signalling in lizards, and suggest that the equilibrium between each sensory modality in each population or species may depend on the interaction social environment and microhabitat characteristics.

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Capítulo 2

EL COMPORTAMIENTO ANTIDEPREDATORIO DEPENDIENTE DE LA CONSPICUIDAD PUEDE COMPENSAR LAS DIFERENCIAS DE COLORACIÓN ENTRE DOS ESPECIES HERMANAS

La selección sexual favorece la utilización de señales conspicuas, mientras que la selección natural (a través del riesgo de depredación) favorece a los individuos más crípticos. Sin embargo, este compromiso podría verse alterado si los machos con una coloración más conspicua pudieran compensarla comportamentalmente, siendo más cautos con los depredadores. En este trabajo hemos explorado si existe una regulación intraespecífica del comportamiento antidepredatorio dependiente de la conspicuidad y si las diferencias en conspicuidad de la coloración sexual que existen entre dos especies estrechamente relacionadas de lagartijas serranas (*Iberolacerta monticola* de Galicia e *I. cyreni* de Guadarrama) podrían mantenerse gracias a la compensación del comportamiento antidepredatorio. Nuestros resultados sugieren que los machos de lagartija pueden compensar los efectos negativos en cuanto a riesgo de depredación de la coloración sexual conspicua modulando su comportamiento antidepredatorio (tiempo dentro del refugio, falsas alarmas, etc.) Hemos encontrado que los machos de *I. monticola*, pero no los machos de *I. cyreni*, compensan los efectos negativos de los ocelos azules laterales, los cuales incrementan su conspicuidad visual. Sin embargo, no compensan los efectos de los ocelos ventrales, los cuales se encuentran relativamente poco visibles. También hemos encontrado que los machos de *I. monticola* en mejor condición y con más ocelos azules laterales fueron más tímidos frente a los potenciales depredadores, mientras que los machos de *I. cyreni* en mejor condición y con más ocelos ventrales fueron más arriesgados. Estas dos especies de lagartijas viven en hábitats que difieren en la disponibilidad de refugios y en el número de potenciales depredadores, lo que podría promover diferencias en el compromiso entre riesgo de depredación y comportamiento social y explicar las diferencias interespecíficas observadas en el comportamiento antidepredador. Esto sugiere que la regulación del comportamiento antidepredador puede también funcionar como un coste dependiente de la condición que promovería la señalización sexual costosa (honesta) en algunas especies ■

CONSPICUOUSNESS-DEPENDENT ANTIPREDATORY BEHAVIOR MAY COUNTERACT COLORATION DIFFERENCES IN IBERIAN ROCK LIZARDS

Sexual selection favors more conspicuous male displays, while natural selection (via predator pressure) favors less conspicuous displays. However, this trade-off might be altered if males with more conspicuous displays could compensate behaviorally for their increased conspicuousness by acting more cautiously towards predators. The aim of this study was to explore in two species of Iberian rock lizards whether or not conspicuous coloration was associated with antipredatory behavior and whether conspicuousness-dependent regulation of antipredatory behavior existed. Our results suggested that male lizards may compensate for the negative effects of conspicuous sexual coloration on predation risk by modulating their antipredatory behavior (time inside refuges, false alarms, etc). We found that male *Iberolacerta monticola*, but not male *I. cyreni*, compensated for the negative effects of blue lateral ocelli, which increased visual conspicuousness. However, male lizards did not compensate for relatively unexposed ventral spots. We also found that male *I. monticola* in better condition and with more blue lateral ocelli were shier, whereas male *I. cyreni* in better condition and with more ventral spots were bolder. These two lizard species live in habitats that differ in refuge availability and in the number of potential predators, which may promote differences in the trade-off between predation risk and social behavior and may explain the observed interspecific differences in antipredatory behavior. This suggests that regulation of antipredatory behavior may also function as a condition dependent cost promoting costly (honest) sexual signaling in some species.

Key words: sexual signals, conspicuous coloration, antipredatory behavior, predation costs, predation risk, lizards.

Sexual selection arises from competition among individuals of one sex for access to mates, giving rise to the evolution of extravagant secondary sexual characters or signals that are used in attraction of mates or in fights for access to mates (Andersson 1994). This raises the question of why males of some species have evolved bright colors, whereas others, even in the same genus, are dull colored. The function and evolution of such variation is usually explained by a balance between sexual selection and species recognition (via female choice), which usually favor conspicuous signals, and natural selection (via predator pressure), which favors cryptic individuals (Endler 1978, 1980; Andersson 1994; Møller and Nielsen 1996; Deutch 1997; Zuk and Kolloru 1998). However, high predation exposure may be compensated for by antipredator behavior (Forsman and Appelqvist 1998; Martín and López 1999b; Losos et al. 2004). Flexible antipredator behaviors are very common across taxa (Lima and Dill 1990). Animals are able to assess predation risk and modify their antipredator behavior depending, for example, on microhabitat characteristics (e.g., Martín and López 1995; Losos and Irschick 1996) or locomotory abilities (Braña 1993; Martín 1996). If males with more conspicuous displays could compensate behaviorally for their increased conspicuousness by acting more cautiously towards predators, the trade-off between sexual and natural selection might be altered. However, the influence of conspicuousness on antipredator behavior remains little explored (but see Eterovick et al. 1997; Forsman and Appelqvist 1998; Hedrick 2000; Cuadrado et al. 2001; Lewkiewicz and Zuk 2004; Lindström et al. 2007).

Iberian rock lizards (*Iberolacerta* spp.) comprise a group of closely related species of medium sized lizards found mainly in rocky habitats in the Iberian Peninsula (Mayer and Arribas 2003; Carranza et al. 2004). The Carpetane rock lizard, *Iberolacerta cyreni* (formerly *Lacerta monticola cyreni*) is found at high altitude mountains in Central Spain (above 1800 m asl and until 2400 m asl) where it reaches higher population densities than the closely related Iberian rock lizard, *I. monticola* (formerly *L. monticola cantabrica*), which is found in lower mountains (below 1500 m asl) and lowland valleys, even at the sea level, in North-western Spain (Galán and Fernández-Arias 1993; Martín 2005a,b). Both species of Iberian rock lizards select microhabitats with high cover of large rocks with crevices that provide refuges (Martín and Salvador 1997; see results) and are typical prey for snakes and mammals and also for many avian predators such us raptors or shrikes (see results; Martín 2005a,b). The lowland lizard species (*I. monticola*) develops more extensive and colorful breeding coloration (i.e., dorsal and ventral bright green coloration, blue spots on lateral-ventral scales and blue ocelli over shoulders) than *I. cyreni* (i.e., dull blue-green coloration and a high number of blue spots on lateral-ventral scales but none or rarely just one blue ocellum over shoulders) (Arribas 2001, Galán 2008). Blue spots and ocelli of lacertid lizards reflect ultraviolet (UV) light (Arribas 2001; Thorpe and Richard 2001) and both lizards and avian predators can perceive the reflectance of UV light (Fleishman et al. 1993; Cuthill et al. 2000). Bright colors render male lizards more conspicuous, possibly resulting in a greater susceptibility to predators (e.g., Martín and López 2001; Stuart-Fox et al. 2003; but see Olsson 1993), thus we expected that a higher number of UV-blue spots increased conspicuousness of Iberian rock lizards, at least, to avian predators. Then, the first goal of this study was to explore whether conspicuousness-dependent regulation of antipredatory behavior in Iberian rock lizards exists, and whether it might explain interspecific differences in coloration/conspicuousness. Between- species differences in conspicuousness of signals or predation risk level, may affect cost of signaling, which may also affect the adaptative value of conspicuousness-dependent regulation of antipredatory behavior. Lizard populations at high elevations enjoy high survivorship and reach higher densities than those at lower elevations (Bashey and Dunham 1997), likely because of reduced predation (Van Damme et al. 1989; Fox et al. 1994), abundant food, and the uniformly distribution and abundance of appropriate thermal microhabitats. We hypothesized that if predation cost of signaling (i.e., high predation risk level) was high, the strength of conspicuousness-dependent regulation of antipredatory behavior may be higher. Typically, it is assumed that two localities differ in predation risk when they differ in predator density or community (McLaughlin and Roughgarden 1989, Van Damme and Castilla 1996), or in the availability of protective cover (Snell et al. 1988). Avian predators rely on visual active searching, whereas snakes and mammals rely mainly on chemical cues to locate prey. Thus, to estimate relative predation risk to visual predators on both species of Iberian rock lizards, we surveyed published studies to know the number of potential avian predator species at each population. We also measured microhabitats available and used by lizards to estimate availability of refuges, and examined whether potential differences in visual backgrounds (vegetation and substrate cover in the microhabitats selected by lizards) may render one species to be more conspicuous than the other against their respective visual backgrounds.

Finally, behavioral compensation of conspicuousness may be costly (condition dependent) and, therefore, may affect by itself costs of signaling. One prominent model of sexual selection, the handicap model, assume that the marginal costs of signaling must be lower for males in good condition than for males in poor condition (Grafen 1990; Rowe and Houle 1996; Tomkins et al. 2004). Since the empirical evidence for direct fitness costs created by predators on sexual signals is scarce (Kotiaho 2001; but see Zuk and Kolluru 1998) it is not well understood whether predation costs increase in proportion to the expression of the signal or whether they are related to the condition of the males (see Candolin 1997, 1998; Kotiaho et al. 1998; Hedrick 2000; Lewkiewicz and Zuk 2004). Therefore, regulation of antipredatory behavior may compensate for the predation costs of increased sexual signalling. If individuals in better condition are better at escaping predation, and if signalling rate is positively dependent on condition, then the condition dependent escape rate would reduce the cost of signalling in males that are signalling the most, thus fulfilling the assumptions of good genes sexual selection theory (Kotiaho 2001).

To examine whether antipredator behavior varied between individuals, within each species, in relation to individual conspicuousness, we simulated predatory attacks to lizards in the laboratory. We predicted that only visual signals that increased conspicuousness to visual predators may promote antipredatory behavior compensation. We used the number of UV-blue shoulder ocelli as a measure of visual conspicuousness, because, in these ground dwelling lizards, these ocelli are clearly visible from above by predators. In contrast, UV-blue ventral spots are less visible from above and we considered them as signals relatively less visible to predators. We hypothesized that if predation cost of signaling (i.e., high predation risk level) was high (due to higher number of predator species or lower number of refuges), the strength of conspicuousness-dependent regulation of antipredatory behavior may be higher. Thus, we explored whether there were between-species differences in the strength of the relationships between conspicuousness and antipredatory behavior. Finally, we also included body condition in our within-species comparisons to test whether interspecific differences may promote differences in condition dependence of conspicuousness-dependent antipredatory behavior.

Methods

Study animals

Between February and April 2005, we captured by harmless noosing 15 recently post-hibernation emerging adult male lizards *I. cyreni* (snout-to-vent length, SVL, mean ± SD = 77 ± 3 mm) in alpine habitat at the Guadarrama Mountains in Madrid

(40°77’N, 4°1’W; 1950 m asl), and 16 adult male lizards *I. monticola* (SVL= 77 ± 2 mm) in moist and vegetated lowland habitat at coastal valleys in Galicia (43°70’N, 7°98’W; 100 m asl). At least two months before testing, to allow acclimation to laboratory conditions and to homogenize previous recent predation risk experience, lizards were individually housed at “El Ventorrillo” Field Station (Navacerrada, Madrid prov.), in outdoor 60 x 40 cm PVC terraria containing rocks for cover. The photoperiod and ambient temperature were those of the surrounding region and water and mealworm larvae dusted with multivitamin powder were provided ad libitum. Experimental lizards were fed ad libitum to ensure top foraging condition and to avoid individual differences in foraging abilities that might distort antipredatory behavior. However, we did not find differences in male body condition between time of capture and time of experiments (Repeated measures ANOVAs, *I. monticola*: $F_{1,11} = 0.0047$, $P = 0.94$; *I.cyreni*: $F_{1,9} = 0.40$, $P = 0.54$), which suggests a lack of food limitation in natural conditions. Thus, although individual differences in body condition could depend on food intake, differences among our experimental individuals seem rather due to physiological requirements mediated by the health dependent “individual quality”, which may influence antipredatory behavior (see López et al. 2005). All lizards were healthy during the trials, and were returned to their exact capture sites at the end of experiments.

We measured SVL and tail length to the nearest 1 mm with a ruler, and counted the number of shoulder ocelli and ventral spots on the right and left side (2–4 counts per animal). Ocelli or spots with bright blue color reflect ultraviolet (UV) light (Arribas 2001; Thorpe and Richard 2001; unpublished data) were classified as “blue shoulder ocelli” or “blue ventral spots”, and small ones or those with dull blue or white coloration (which do not reflect UV; unpublished data) were classified as “dull shoulder ocelli” or “dull ventral spots”.

Antipredator behavior

We studied antipredator behavior of male lizards (*I. cyreni*: $N = 15$; *I. monticola*: $N = 16$) in outdoor conditions during July and August, from 1100 to 1400 hours GMT, when lizards were fully active. We allowed lizards to thermoregulate and attain their preferred body temperatures for at least 1 h before the trials (Martín and Salvador 1993). Terraria were placed separately from each other, such that our approaches to a terrarium did not influence lizards in other terraria.

To examine antipredator responses, we previously noted whether the lizard was hiding inside the refuge, leaning out of the refuge (i.e., the lizard stayed inside the refuge but looked outside with the snout closer than 1 cm to the exit of the refuge) or outside the refuge, and then walked slowly, stopped close (50 cm) to each terrarium, and looked directly to the lizard during ten seconds, verifying that lizards could clearly see the experimenter from their terraria. The same person performed all predatory threats. We simulated 16-18 predatory attacks to each lizard within a day (one every 10 min within 3 h), which we repeated over 24 h later. From the observations of the initial position of lizards taken every 10 min immediately before each approach, we calculated the average number of times that a lizard was outside, inside or leaning out of the refuge, which were considered as a measure of the antipredator behavior of lizards to the previous approaches of the experimenter. We also noted whether the lizard hid in the refuge when the experimenter stopped close to the terrarium and looked directly inside it. We considered that if a lizard hid, it perceived a high predation risk, given that the predator was looking to but not actually attacking the lizard. Although responding to all approaches by fleeing to the refuge would minimize predation risk, time and energy can be saved, and costs of refuge use minimized, if lizards responded accurately only to actual predatory attacks (Ydenberg and Dill 1986; Martín and López 1999a). Thus, we calculated the proportion of times that a lizard that was initially outside or leaning out of the refuge hid ('false-alarms'), and the proportion of times that was initially outside the refuge and remained outside ('undisturbed') after the experimenter approached. We considered that a high number of "false-alarms" indicated shyness while a low number indicated boldness (López et al. 2005). With this procedure we simulated a threat of a predator coming from above the lizard because it is likely that lizards respond to human beings as if they were visual predators (Cooper 2003). We predicted that more conspicuous individuals should respond to simulated predatory attacks with shyer antipredatory behavior (higher proportion of time inside the refuge and higher number of "false-alarms") while less conspicuous individuals should respond with bolder antipredatory behavior (more time outside the refuge and lower number of "false-alarms") (López et al. 2005). To test for possible effects of habituation to simulated predator attacks, we tested for individual consistency in antipredator behaviour between both days. Repeatability was calculated as the intraclass correlation coefficient based on variance components derived from an one-way ANOVA (Lessells and Boag 1987). All antipredator behavioral variables were significantly repeatable in both days ($0.43 < r < 0.82$, $0.04 > P > 0.0001$ in all cases) except "time leaning out the refuge" ($P > 0.08$). Thus, for the subsequent analyses, we discarded this variable and used the average values of the two days for all the other variables.

To examine the possible relations between the average values of the antipredator behavior variables (time inside refuge; false-alarms; undisturbed) and number of

Table 1. Relationships between antipredatory behavioral variables and visual sexual signals or body condition in male lizards

	Time inside refuge	Time outside	False alarms	Undisturbed
I. monticola	$F_{1,12}$ β P		$F_{1,13}$ β P	
Blue shoulder ocelli	**10.12 0.85 0.007**	*ns*	*ns*	*ns*
Dull shoulder ocelli	*ns*	*ns*	*ns*	*ns*
Blue ventral spot	*ns*	*ns*	2.35 −0.34 0.15	*ns*
Dull ventral spot	*ns*	*ns*	*ns*	*ns*
Condition	*ns*	*ns*	4.38 −0.46 0.056	*ns*

I. cyreni			$F_{1,10}$ β P	$F_{1,11}$ β P
Blue shoulder ocelli	*ns*	*ns*	*ns*	*ns*
Dull shoulder ocelli	*ns*	*ns*	*ns*	*ns*
Blue ventral spot	*ns*	*ns*	**58.56 −2.47 .001**	**6.91 1.46 0.023**
Dull ventral spot	*ns*	*ns*	**53.82 2.36 .001**	**6.34 1.45 0.028**
Condition	*ns*	*ns*	**9.40 0.40 0.011**	**6.69 0.60 0.025**

P and β values from univariate regressions. Significative correlations are showed in bold; *ns* = not significative shoulder ocelli and ventral spots reflecting conspicuousness of lizards (all variables log transformed) we developed GLMs (using Statistica 6.0 software) within each species separately. Data normality was verified by Shapiro-Wilk's tests, and tests of homogeneity of variances (Leveane's test) showed that variances were not significantly heterogeneous after transformation. However, lateral ocelli in *I. cyreni* did not fit the assumptions of normality and were analyzed with Spearman's rank order correlations. We did not compare the two species in a single analysis because interspecific differences in ocelli and spots numbers did not comply with the assumption of homogeneous slopes between groups (Engqvist 2005). We also included body condition (calculated as the residuals from the regression equation of body mass (g) on SVL (mm), both variables log transformed) in the models because behavioral responses might be related to differences in the physical condition of otherwise similar individuals. Body condition of each individual may represent an index of the relative amount of fat stored, and, hence, of individual physical condition or nutritional status (reviewed in Green 2000). To decide what variables best predicted antipredator behavior of males, we used a "best-subsets" approach in conjunction with stepwise methods (Neter et al. 1985). We chose the most parsimonious model as having the lowest Akaïke information criterion (AIC), and we checked its coincidence with the equivalent stepwise solution. Residuals from the final models were normally distributed (Grafen and Hails 2002).

Sympatric avian predator species

To obtain a rough between-species comparison of numbers of bird species that are known to prey on Iberian rock lizards, we compiled a list of potential predator species based on published studies, most of them made in the same area or very close geographical area (Martín and López 1990; Salvador and Veiga 2003 Martín 2005a,b; Martí and del Moral 2003). We also obtained from published sources (Martí and del Moral 2003) data on the presence and densities of these predators in both study sites.

Microhabitat selection

We measured microhabitat use by lizards to obtain a between-species comparison of refuge and visual background (vegetation and substrate cover) selection and availability. Visual background was examined because although both species differ in the number of blue shoulder ocelli and ventral spots, this might result in equal conspicuousness if visual background were different.

We searched for lizards by walking during April between 07:00 and 14:00h (GMT) with consistent sampling effort in the two study sites. We recorded microhabitat data at the point where each individual was first sighted (*I. cyreni*: n = 29; *I. monticola*: n = 21). Four 1 m transects were laid out radiating from this point along the four cardinal directions, and records made at 5, 10, 15, 25, 75 and 100 cm. We noted the presence at substrate level of grass, leaf litter, bare sandy soil, small rocks (< 25 cm), medium rocks (25-100 cm), large rocks (100-200 cm), or rocky outcrops and cliff walls (> 200 cm). Plant contacts at 5, 10, 25 and 50 cm height were also noted, using a calibrated stick, for grasses or small herbaceous semi-perennial plants (<50 cm height), large woody bushes (*Cytisus oromediterarneus* and *Juniperus comunnis* in Guadarrama, or *Cytisus scoparius* and *Ulex europaeus* in Galicia) or trees (*Pinus sylvestris*). We also noted whether the sample point was in a sunny or shaded location, the presence of canopy tree cover above each point, and the distance to the nearest potential refuge (rock crevice or protective vegetation cover). This procedure provided 24 sample points per lizard location and allowed us to calculate the percent cover values of each habitat variable (i.e., % contacts with each substratum, vegetation type and sunny spots, and the mean distance to the nearest refuge; for a similar sampling methodology see Martín and Salvador 1997). To estimate availability of microhabitats in a large area surrounding that actually used by lizards, we used a similar procedure to record the same variables as described above at 2, 3 and 4 m. along the four cardinal directions from each sample point.

We used a principal component analysis (PCA) to reduce the microhabitat variables to a smaller number of independent components. Only principal components (PCs) with eigenvalues that explained more than 5% of the total variation and had biological significance were retained for further analysis. Original data (number of contacts) were normalized by means of square-root transformation. Because of the large number of "zero" observations for many variables, some transformed data were not normally distributed, but otherwise met the assumptions of a PCA. Deviations from normality do not necessarily bias the analysis, as long as the distributions are reasonably unskewed (Legendre and Legendre 1998). Thus, we considered that our transformations reduced the skew sufficiently to minimize the risk of bias in the analysis. We used general linear models (GLM) to compare PC scores describing microhabitat characteristics in relation to each species (*I. monticola* vs. *I. cyreni*) and the type of point (available vs. used by lizards) to determine whether lizards used available microhabitats in a non-random fashion. We included the interaction between species and type of point in the model to test for differences in microhabitat selection of lizards within each species. Post-hoc pairwise comparisons were planned using Tukey's honestly significant difference tests (Sokal and Rohlf 1995).

Results

Antipredator behavior

Separated analyses within each species showed that positive conspicuousness-dependent regulation of antipredatory behavior may occur in male *I. monticola* but not in male *I. cyreni* (Figure 1; Table 1). Thus, in male *I. monticola*, the best model based on AIC for time spent inside the refuge included blue shoulder ocelli, dull shoulder ocelli and body condition. A stepwise multiple regression analysis (GLM model: adjusted R^2 = 0.47, $F_{3,12}$ = 5.46, P = 0.013) confirmed that time spent inside the refuge was positively and significantly correlated with the number of blue shoulder ocelli (Table 1). For the number of false alarms, the best model based on AIC included blue ventral spots

Table 2. Antipredatory behavior variables of lizards

	I. monticola (mean ± SE)	*I. cyreni* (mean ± SE)	$F_{1,29}$	P
Inside the refuge (%)	63.0 ± 4.0	39.6 ± 5.7	11.80	0.001
Leaning out of the refuge (%)	26.0 ± 3.6	5.5 ± 1.2	0.25	0.61
Outside the refuge (%)	4.7 ± 1.0	49.4 ± 5.9	11.59	0.001
False alarms (%)	30.1 ± 3.7	13.8 ± 2.2	42.2	< 0.001
Undisturbed (%)	2.3 ± 1.0	40.2 ± 4.7	52.75	< 0.001

F and *P* values from protected one-way ANOVA tests and body condition, and stepwise multiple regression analysis (GLM model: adjusted R^2 = 0.35, $F_{2,13}$ = 5.18, P = 0.022) showed that blue ventral spots were not significantly correlated, and the negative correlation with body condition approached significance (Table 1). Thus, male *I. monticola* with a higher number of conspicuous ocelli (shoulder ocelli) spent less time exposed to predator threats. Likewise, male *I. monticola* with worse body condition retreated early into refuges under a predator threat. Visual signals no exposed to predators (ventral spots) were not significatively correlated with any behavioral variable (P > 0.12 in all cases).

In contrast, visual signals exposed to predators (shoulder ocelli) of male *I. cyreni* did not significantly correlate with any behavioral variable (Spearman's rank correlation: $-0.28 < r_s < 0.27$, $P > 0.30$ in all cases)(Figure 1). However, signals not exposed to predators (lateral ocelli) correlated with bolder behavior (Table 1). The best stepwise multiple regression model based on AIC for the number of false alarms included dull shoulder ocelli, blue ventral spots, dull ventral spots and body condition (GLM model: adjusted R^2 = 0.80, $F_{4,10}$ = 15.16, P < 0.001) showing that the number of false alarms was negatively and significantly correlated with the number of blue ventral spots and positively and significantly correlated with the number of dull ventral spots and with body condition (Table 1). The best stepwise multiple regression model based on AIC for the proportion of times that male *I. cyreni* remained undisturbed included blue ventral spots, dull ventral spots and body condition (GLM model: adjusted R^2 = 0.35, $F_{3,11}$ = 3.59, P = 0.049), showing that the proportion of times that male *I. cyreni* remained undisturbed was positively and significantly correlated with the number of blue ventral spots and negatively and significantly correlated with the number of dull ventral spots and with body condition (Table 1). Thus, male *I. cyreni* in better condition retreated more often into the refuge (i.e., higher number of false alarms) and remained less often undisturbed under a predator threat.

Multivariate analyses on the antipredatory behavioral variables showed that there were significant differences between the two lizard species (MANOVA, Wilks' λ = 0.20, $F_{5,25}$ = 18.89, P < 0.001). Univariate protected ANOVAs showed that these were due to significant differences in all behavioral variables, except in "time spent leaning out of the refuge" (Table 2). Thus, male *I. monticola* spent more time inside the refuge, performed a higher number of false alarms and remained less time undisturbed than male *I. cyreni*.

Between-species differences in UV-signals

Male *I. monticola* had a significantly larger number of blue and dull shoulder ocelli (mean of right + left sides) than male *I. cyreni* (blue shoulder ocelli: mean ± SD = 2.2 ± 0.9 *vs* 0.8 ± 1.0; Mann-Whitney U test, Z = 3.20, n_1 = 16, n_2 = 15, P = 0.001; dull

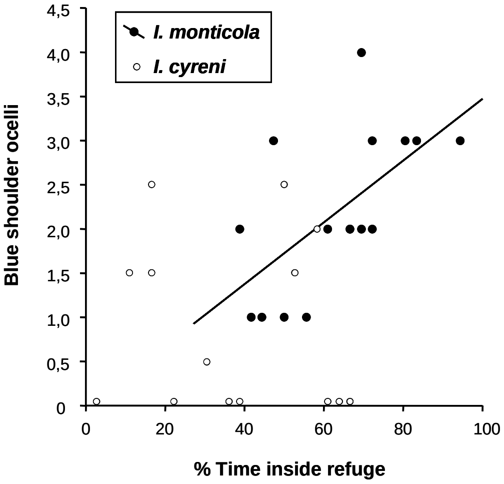

Figure 1. UV-blue shoulder ocelli (exposed sexual signal) versus time spent inside the refuge after simulated predatory threat for male *I. monticola* (solid circles) and male *I. cyreni* (open circles). Regression line is showed for male *I. monticola*; UV-blue shoulder ocelli did not correlate significantly with time inside refuge for male *I. cyreni*.

shoulder ocelli: 1.2 ± 0.7 *vs* 0.4 ± 0.5; $Z = 3.02$, $n_1 = 16$, $n_2 = 15$, $P = 0.002$). In contrast, male *I. monticola* had significantly less unexposed blue and dull ventral spots (mean of right + left sides) than male *I. cyreni* (blue ventral spots: 1.3 ± 1.6 *vs* 8.1 ± 3.6; $Z = -4.13$, $n_1 = 16$, $n_2 = 15$, $P < 0.001$; dull ventral spots: 1.5 ± 1.6 *vs* 3.7 ± 1.4; $Z = -3.24$, $n_1 = 16$, $n_2 = 15$, $P = 0.001$).

Table 3. Potential predator species of lizards in both study sites and densities (individuals/Km^2) of avian species

	I. monticola	*I. cyreni*
Raptors:		
*Milvus migrans**	0-1	0
Accipiter gentilis	0-1	0
Accipiter nisus	0-1	0
Buteo buteo	**1-2**	0
Circaetus gallicus	0-1	0
Hieraetus pennatus	0	0-1
*Circus pygargus**	0-1	0
Falco tinnunculus	**1-2**	0
Gulls:		
Larus michahellis	20-200	0
Shrikes:		
Lanius collurio	**1-2**	0
Thrushes:		
*Monticola saxatilis**	0-1	1-2
*M. solitarius**	0-1	1-2
*Turdus merula**	20-200	0
Crows:		
Pica pica	**2-20**	0
Corvus corax	0-1	**1-2**
Corvus corone	**20-200**	0

* = occasional predator of lizards. Species that implies higher predation risk (regular predators of lizards with higher densities) are highlighted in bold.

Between-species differences in sympatric avian predator species

The two species of Iberian rock lizards live in habitats that differ in the number of potential avian predators. In the lowland habitat of *I. monticola*, potential avian predators are seven raptor species, one gull, one shrike, three thrushes and three crows (Table 3). In contrast, at the highland locality of *I. cyreni*, potential avian predators are only one raptor, two thrushes and one crow (Table 3). Moreover, the densities of regular avian predators are higher in the habitat of *I. monticola* (Table 3).

Between-species differences in microhabitat characteristics

The PCA for microhabitats available and those used by lizards produced three components that together accounted for the 56.62 % of the variance. The first PC (PC-1, eigenvalue = 5.02, 26.43 % of total variance) was negatively correlated with sunny locations ($r = -0.51$) and positively correlated with the presence at substrate level of leaf litter ($r = 0.64$) and contacts with large woody bushes at 5, 10, 25, 50, 75, 100 cm height ($0.76 < r < 0.84$). The second PC (PC-2, eigenvalue = 3.01, 15.89 % of variance) was negatively correlated with cover of large rocks ($r = -0.49$), rocky outcrops ($r = -0.55$) and distance to the nearest potential refuge ($r = -0.79$) and positively correlated with cover of small ($r = 0.57$) and medium rocks ($r = 0.71$). The third PC (PC-3, eigenvalue = 2.71, 14.29 % of total variance) was negatively correlated with cover of bare sandy soil ($r = -0.47$), grass contacts at 5, 10, 25 cm height ($-0.85 < r < -0.69$) and tree cover ($r = -0.58$).

There were significant differences in relation to all PCs between the two lizard species (GLM, Wilks' $\lambda = 0.25$, $F_{3,94} = 92.24$, $P < 0.0001$) and between types of microhabitat points (available *vs.* used by lizards; Wilks' $\lambda = 0.63$, $F_{3,94} = 18.30$, $P < 0.0001$) (Figure 2). The interaction between species and type of point was significant (Wilks' $\lambda = 0.68$, $F_{3,94} = 14.33$, $P <$

0.0001). The general model showed significant overall differences for all PCs (PC-1: adjusted $R^2 = 0.24$, $F_{3,96} = 11.61$, $P < 0.0001$; PC-2: adjusted $R^2 = 0.58$, $F_{3,96} = 49.50$, $P = 0.0001$; PC-3: adjusted $R^2 = 0.06$, $F_{3,96} = 3.40$, $P = 0.02$). There were significant differences between populations in characteristics of available microhabitats defined by PC-1 and PC-2 scores (Tukey's tests, $P < 0.001$ in both cases), but not in those defined by PC-3 ($P = 0.91$). Thus, in the habitat of *I. monticola* there was a higher cover of large woody bushes with presence at substrate level of leaf litter and large rocks or rocky outcrops, whereas in the habitat of *I. cyreni* there was a higher cover of small and medium rocks, with many crevices, and refuges were closer. Regardless of these differences in availability, there were not significant differences between species in the PC-1 and PC-3 scores of microhabitat used by lizards (Tukey's tests, $P > 0.60$ in both cases), but there were significant differences between species in the PC-2 ($P < 0.001$). Thus, males of both species used microhabitats with more sunny locations and less cover of small woody bushes, trees and grasses, but *I. cyreni* used microhabitats with more small and medium rocks and closer to refuges than *I. monticola.* In sum, microhabitats available to the two species did not differ in characteristics such as the extent of vegetation or substrate cover, which may affect the visual background. The species selected microhabitats that differed in refuge availability or distance to the nearest refuge; however, neither of these factors are likely to affect the visual background and therefore should not affect relative conspicuousness.

There were not significant differences between microhabitats available and used by *I. cyreni* in all PC scores (Tukey's tests, $P > 0.10$ in all cases), but there were significant differences between microhabitat used by *I. monticola* and microhabitat availability in PC-1 and PC-2 scores ($P < 0.001$ in both cases) but not in PC-3 ($P = 0.91$). Thus, microhabitat availability and microhabitat preferences corresponded in *I. cyreni*, whereas *I. monticola* selected microhabitats with more sunny locations and less cover of small woody bushes (PC-1) and with more small and medium rocks and closer to refuges (PC-2) than those available in their habitat.

Discussion

Increased predation pressure may shape the evolution of sexual traits favoring less conspicuous males (Endler 1982; Candolin 1998) or with colors biased against the spectral sensitivity of the most dangerous predator (Endler 1991). However, our results suggested that male lizards may compensate for negative effects of conspicuous sexual coloration on predation risk by modulating antipredatory behavior. This conspicuous-dependent antipredatory behavior may also function as a condition dependent cost promoting costly (honest) sexual signaling in some species but not in others.

Conspicuousness-dependent regulation of antipredatory behavior

Behavioral compensation has been reported in displaying males (Ryan et al. 1982; Hedrick 2000; Lewkiewicz and Zuk 2004; Lindström et al. 2007) but, to our knowledge, whether brighter coloration may be behaviorally compensated has been scarcely studied in vertebrates (but see Martín and López 1999b; Cuadrado et al. 2001). We found that more conspicuous individual male *I. monticola* behave more cautiously; hiding for longer in refuges when they were threatened by a potential predator. Predation pressure may act differentially for exposed or unexposed signals. For example, body regions of lizards hidden from predators are more chromatically contrasting against the background than body regions exposed to predators (Stuart-Fox et al. 2004). Thus, we predicted that lizards should compensate for the negative effects of signals that increase conspicuousness to predators (lateral ocelli), but not for unexposed signals (ventral spots). In accordance, we found that male *I. monticola* with more shoulder ocelli, but not ventral spots, behaved shyer to compensate for their increased conspicuousness. Previous studies have shown that refuge use of Iberian rock lizards may vary as a function of predation risk level, physiological costs and foraging or mating expectations outside refuges (Martín and López 1999a; Martín et al. 2003a, b). The compensation for conspicuousness could alter the trade-off between sexual and natural selection, thereby changing the dynamics of evolutionary models that assume that conspicuous male characters carry a cost in terms of natural selection. Thus, males with conspicuous colorations might not always experience as greater actual predation rates as suggested by some studies that used artificial static replica models of lizards (e.g., Stuart-Fox et al. 2003; Husak et al. 2006).

We did not found any correlation between antipredatory behavior and exposed signals in male *I. cyreni,* but a higher number of UV-blue ventral spots predicted bolder antipredator behavior. Most studies show that visual conspicuousness of male coloration correlates positively with shyness (Forsman and Appelqvist 1998; Martín and López 1999b; Hedrick 2000; Cuadrado et al. 2001; Lindström et al. 2007) but there are some exceptions (Godin and Dugatkin 1996). The non adaptative or weaker value of conspicuousness-dependent regulation of antipredatory behavior when cost of signaling is low (because of reduced predation risk and low signal conspicuousness) may explain the lack of conspicuousness-dependent regulation of antipredatory behavior in some species. The UV-blue ventral spots of *I. cyreni* are hidden to predators, so they should not increase predation exposure (Stuart-fox et al. 2004).

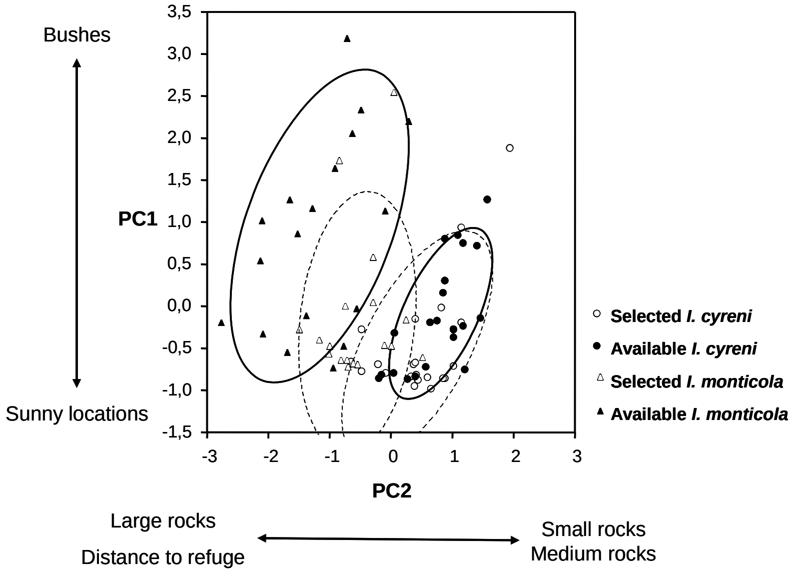

Figure 2. Microhabitat selected and available by male *I. monticola* and male *I. cyreni*, based on the two first PC scores representing 24 microhabitat characteristics; 95% confidence ellipses are shown.

Moreover, our results of microhabitat selection suggested lower predation risk on *I. cyreni*, which is expected to occur in montane lizards (Van Damme et al. 1989; Fox et al. 1994). Differences in protective cover or the number of predator species affect predation risk (Snell et al. 1988; McLaughlin and Roughgarden 1989) and we found that the number and densities of avian predators were higher in the habitat of *I. monticola* which also showed a higher number of exposed UV-blue shoulder ocelli. Ornaments with bright and UV coloration may increase predation risk because diurnal raptors (Viitala et al. 1995, Honkavaara et al. 2002) and shrikes (Probst et al. 2002) use UV wavelengths as a hunting cue, so between-species differences in avian predators are especially important. Therefore, more conspicuous male *I. monticola* may spent more time inside refuges because their risk of be detected by a predator was higher than for more conspicuous male *I. cyreni*. We also found that in the habitat of *I. monticola* refuges were far and scarce, which may implicate high risk of predation and force lizards to select specific microhabitats where refuges are close. In contrast, male *I. cyreni* did not choose particular microhabitats because the high availability of small to medium rocks in their habitat provided abundant, evenly distributed and close refuges. Moreover, both species selected microhabitats with the same cover of vegetation (grasses or small woody bushes) and used the same class of granite rocks for thermoregulation and refuge (personal observation) so we may expect that both species have similar visual backgrounds and visibility and that the number of UV-blue shoulder ocelli may be correlated with actual conspicuousness of Iberian rock lizards in their native habitats.

Although our study clearly suffers from the potential problems of inferring adaptation from two-species comparisons (Garland and Adolph 1991), our within species comparisons suggest that differences in antipredator behavior may be causally related to differences in predation costs of signals. Moreover, both lizard species are closely related and until recently were considered as belonging to the same species (Mayer and Arribas 2003; Carranza et al. 2004), so that any effect due to phylogenetic divergence should be very weak. Thus, our results may support the hypothesis about the adaptative value of conspicuousness-dependent regulation of antipredatory behavior only when cost of signaling was high (Kotiaho, 2001), which may occur in *I. monticola*, but not *I.cyreni*. The habitat of *I. monticola* has greater predation risk and fewer available shelters. Despite this, *I. monticola* is more conspicuous, potentially indicating stronger sexual selection on coloration in this species. These factors combined are likely to explain shyer behaviour as well as behavioural compensation in *I. monticola* but not in *I. cyreni*. Similarly, male lacertid lizards *Gallotia galloti* have more exposed UV blotches in habitats where they are more vulnerable to predation (Thorpe and Richard 2001).

In addition, for male *I. cyreni,* antipredatory behavior suppose to lose relatively more mating and thermoregulatory opportunities (see Martín et al 2003a) than for male *I. monticola* due to overall differences in mating season duration. Activity level has a consistent effect on reproductive success in *I. cyreni* (Salvador et al. 2008) and bolder behavior may be correlated with higher activity level. In *I. cyreni*, activity of individual males was correlated with their social rank (Martín and López 2000). More active males gained access to more females by overlapping not only home ranges of a larger number of females but also those of a larger number of male competitors, which increased the frequency of agonistic interactions (Aragón et al 2001). Then, the bolder behavior of male *I. cyreni* might be an attempt to balance predation risk with mate searching and thermoregulation (Martin et al 2003a). UV-blue ventral spots may be used to signal size or body condition and during agonistic encounters the presence of blue spots may elicit aggressiveness (López et al 2004). Both activity and sexual signals seem to be subject to hormonal regulation, probably mediated by testosterone, and may have an important effect on reproductive success. Then, if these unexposed signals do not need to be behaviorally compensated to reduce predatory costs, this may explain the positively correlation with bolder behavior.

Condition dependence of antipredatory behavior

Both the energetic and conspicuousness costs of signals have been suggested that may be important to consider in other lizards (Simon 2007) and our data also suggest that conspicuousness-dependent regulation of antipredatory behavior may also function as a condition dependent cost promoting honest sexual signaling in *I. monticola* but not in *I. cyreni*. One prominent model of sexual selection, the handicap model, assume that the marginal costs of signaling must be lower for males in good condition than for males in poor condition (Grafen 1990). If individuals in better condition are better at escaping predation, the condition dependent escape rate would reduce costs of signaling. Male *I. cyreni* with worse body condition were bolder, i.e., had less false alarms, and were more often undisturbed. On the contrary, male *I. monticola* with better body condition performed less "false alarms". This suggests that both species might experience different costs associated to antipredatory behavior. Individuals should optimize their antipredatory responses by balancing antipredator demands with other requirements (Lima and Dill 1990). Between-species differences in mating system or mating season duration may promote differences in the trade-off between predation risk and social behavior (Lima and Dill 1990; Martín et al 2003a).

Due to the alpine environment of *I. cyreni* (high local population densities, extensive home range overlap, and a short breeding season), both body condition and activity level, which may enable males to search more intensively for females, are significant predictors of reproductive success (Salvador et al. 2008). This mating system would allow the coexistence of alternative mating strategies, as suggested by the successful matings obtained by putative ‘‘transients’’ and by the fact that large males did not monopolize females (Salvador et al. 2008). Thus, males in worse condition may gain opportunities to mate by increasing their activity level when predation risk was high and males in better condition were hidden. Concurrently, males with higher reproductive success, presumably males with high activity levels and better body condition, have higher survivorship (Salvador et al. 2008). Thus, although male *I. cyreni* with better body condition may be able to afford the energetic costs of higher activity, their shyer antipredatory behavior may result in higher survivorship without compromising their reproductive success (Abell 2000). Interestingly, this result contrasts with the data available for other lacertids from lower altitudes, such as *Psammodromus algirus*, in which more active males courted females more frequently, but at the cost of decreased survivorship (Díaz 1993; Salvador et al. 1996). Similar relationships might be found in *I. monticola*. Male *I. monticola* may be more territorial than male *I. cyreni* because we found that some microhabitat resources (refuge and sunny locations) differ between selected and available microhabitat in the former species. Moreover, overlapping among male home ranges is lower in *I. monticola* (Moreira et al. 1998) than in *I. cyreni*

(Aragón et al. 2004). Our results showed that male *I. monticola* with better body condition, which may be more territorial, performed less “false alarms” which might allow them to remain more time defending their territories against other males. Thus, condition dependent escape behavior might allow male *I. monticola* to remain more time exposed to predators before hiding. Alternatively, bolder individuals may be in better condition simply because they can spend more time foraging.

Conclusions

A recent study (Oufiero and Garland 2007) illustrates the perils of ignoring potential compensatory mechanisms when studying the costs of sexually selected traits. However, most examples found that lizard populations that experience higher rates of predation exhibit less conspicuous sexual coloration (Macedonia et al. 2002; Kwiatkowski 2003; Stuart-Fox et al. 2004) and that conspicuous males suffer higher predation risk (e.g., Stuart-Fox et al. 2003; Husak et al. 2006; but see Olsson 1993). Most studies on lizards examine predator attacks on static painted models and, therefore, ignore potential compensatory antipredatory behavior. However, our results suggest that conspicuousness-dependent regulation of antipredatory behavior may explain the persistence of brighter signals in species that suffer high predation risk.

By altering the predation cost of sexual signals, conspicuousness-dependent regulation of antipredatory behavior can differentially influence the evolution of honest sexual signalling in closely related species. Ignoring this may lead to erroneous conclusions about the cost of sexually selected traits.

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Capítulo 3

EL ATRACTIVO SEXUAL QUÍMICO DE LOS MACHOS DE LAGARTIJA CANTÁBRICA REFLEJA SU INTERÉS OLFATIVO EN LAS SERPIENTES QUE DEPREDAN EN BASE A SEÑALES QUÍMICAS

Las variaciones en la conspicuidad sexual suelen explicarse como un compromiso entre la selección sexual y la selección natural, a través de la presión depredadora. Sin embargo, la exposición ante los depredadores puede ser compensada mediante comportamientos antidepredatorios. Esta compensación comportamental de la conspicuidad ha sido examinada en distintas especies animales con diferentes sistemas sensoriales, pero nunca se ha mostrado que los machos que producen unas señales químicas más conspicuas para atraer a las hembras compensen comportamentalmente su incrementada exposición ante los depredadores que usan esas señales químicas para localizar a sus presas. Hemos analizado la composición de las secreciones femorales de machos de lagartija cantábrica (*Iberolacerta monticola*), realizado pruebas de asociación de las hembras a los estímulos químicos o visuales de los machos y usado el número de extrusiones linguales que éstos dirigían al olor de una culebra saurófaga y sintópica como medida de su interés en un depredador químico. Encontramos que los machos de lagartija cantábrica con mayor "atractivo químico" para las hembras también mostraban un mayor interés en los estímulos del depredador químico, lo cual sugiere una compensación individual del incrementado riesgo de depredación, mediada por la propia conspicuidad química. Finalmente, se discute cómo la compensación comportamental de la conspicuidad química podría explicar la persistencia de las señales químicas o del marcaje químico. Esto es ignorado en muchos estudios sobre depredación, lo cual puede llevar a conclusiones erróneas sobre el coste de las señales químicas ■

CHEMICAL SEX APPEAL OF MALE CANTABRIAN ROCK LIZARDS MIRRORS THEIR OLFACTORY INTEREST ON CHEMICALLY-GUIDED SNAKE PREDATORS

Variation in sexual conspicuousness is usually explained by a balance between sexual selection and natural selection via predator pressure. However, predation exposure may be compensated for by antipredator behavior. This behavioral compensation of conspicuousness has been examined in several animals using several signaling modalities, but it has never been reported whether individual males that produce more conspicuous chemical signals for attracting females could compensate behaviorally for their increased conspicuousness to chemically-guided predators. We analyzed chemical composition of femoral gland secretions of male lizards *Iberolacerta monticola*, performed female association trials to chemical or visual stimuli of males to determine males' chemical and visual attractiveness, and used tongue flicking (TF) rates of male lizards to the scent of a saurophagous syntopic snake as a measure of interest on a chemically-guided predator. We found that individual male lizards *I. monticola* with higher 'chemical sex appeal' for females also showed more interest on scent of chemically-guided snake predators, suggesting individual compensation for an increased predation risk mediated by their own chemical conspicuousness. We discuss whether behavioral compensation of chemical conspicuousness may explain the persistence of conspicuous chemical signals or scent marking and how most studies on chemical mediated predation ignore potential compensatory antipredatory behavior, which may lead to erroneous conclusions about the cost of chemical signals.

Key words: sexual signals, chemical signals, pheromones, conspicuousness, sexual attractiveness, predation costs, predation risk, lizards.

Sexual signals conspicuous to conspecifics are often also conspicuous to potential predators (Darwin 1871; Verrell 1991; Endler 1992). Variation in sexual conspicuousness is usually explained by a balance between sexual selection and species recognition (via female choice), which usually favor conspicuous signals, and natural selection (via predator pressure), which favors cryptic individuals (Endler 1978, 1980; Andersson 1994; Møller and Nielsen 1996; Zuk and Kolluru 1998). However, predation exposure may be compensated for by antipredator behavior (Forsman and Appelqvist 1998; Martín and López 1999b; Losos et al. 2004; Cabido et al. 2009). For instance, potential victims may reduce predation risk by signaling in areas where (or at times when) detection by predators is minimized (Endler and Théry 1996), or switch courtship tactics depending on perceived predation risk (Endler 1987; Magurran and Seghers 1990; Godin 1995). Thus, animals are able to assess predation risk level and modify their antipredator behavior depending, for example, on microhabitat characteristics (e.g., Martín and López 1995; Losos and Irschick 1996) or locomotory abilities (Braña 1993; Martín 1996).

Males with more conspicuous displays may also compensate behaviorally for their increased conspicuousness by acting more cautiously towards predators, altering the trade-off between sexual and natural selection (Tuttle and Ryan 1982; Lindström et al. 2007; Cabido et al. 2009). This behavioral compensation of conspicuousness has been examined in a wide range of taxa using several signaling modalities, especially the visual (e.g., Forsman and Appelqvist 1998; Cuadrado et al. 2001; Cabido et al. 2009) and acoustic ones (e.g., Tuttle and Ryan 1982; Ryan 1985; Eterovick et al. 1997; Hedrick 2000; Lewkiewicz and Zuk 2004; Lindström et al. 2007).

Chemical signals play an important role in intraspecific communication and sexual selection in many animals, including invertebrates, mammals and reptiles (Mason 1992; Wyatt 2003). Chemical signaling can also have a negative effect by advertising an individual's presence and location to predators (Zuk and Kolluru 1998; Koivula and Korpimäki 2001; Rossell and Sanda 2006) and natural selection should favor incorporating predation risk into the decision-making process of when and where to scent mark (Roberts et al. 2001). Some animals may change their scent marking behavior when they detect a predator (Roberts et al. 2001; Rossell and Sanda 2006). However, it is unknown whether individual males that produce more conspicuous chemical signals for attracting females could compensate for their increased conspicuousness by behaving more cautiously when a chemically-guided predator is detected.

Some studies failed to provide any evidence that animals decrease their frequency of scent marking in the field or in the laboratory in response to the odor of a predator (Wolff 2004), which has been explained because scent marking may have greater benefits in terms of sexual selection than predatory costs (Greenfield 1981; Boake et al. 1996; Wolff 2004). An alternative explanation might be that individuals can modulate antipredatory behavior to compensate for their increased chemical conspicuousness and, then, can maintain their signaling frequency. Thus, the goal of this study is to explore whether antipredatory behavior towards chemically-guided predators may be regulated according to chemical conspicuousness in a species with chemical sexual signaling.

Male lizards of many species possess holocrine epidermal glands that, during the breeding season, deliver secretions used in intraspecific communication (Mason 1992; Alberts 1992a,b). Both the protein (Alberts et al. 1993) and lipid (Alberts 1990; López and Martín 2005) fractions can provide the basis for chemical communication. For instance, secretions from femoral glands allow self-recognition, familiar discrimination and scent-marking of territories (Alberts 1993; Alberts and Werner 1993; Aragón et al. 2001). Lizard femoral secretions, through both productivity rates and/or the quality of the secretions, might also signal male's dominance status (Alberts et al. 1992; Moreira et al. 2006; Martín et al. 2007) or a male's quality, which females may use to select potential mates (Martín and López 2000, 2006; Olsson et al. 2003).

The Cantabrian rock lizard (*Iberolacerta monticola*; formerly *Lacerta monticola cantabrica*) is a small lacertid lizard found in lower mountains (below 1500 m asl) and lowland valleys in North-western Spain (Galán and Fernández-Arias 1993; Martín 2005), where overlap broadly with the smooth snake (*Coronella austriaca*), a saurophagous specialist that typically prey on *I. monticola* and other lizards (Rugiero et al. 1995; Barbadillo et al. 1997). Predators that have coevolved with their prey exploit signal conspicuousness to locate them (Zuk and Kolluru 1998; Ryan et al. 1982) and it is well-established that lizard chemical signals may attract smooth snakes (*Coronella austriaca*), which use them to locate prey (Amo et al. 2004a). Thus, we expected that chemical sex appeal of male *I. monticola* should be related with the risk of been located and preyed by *C. austriaca* snakes. According to the threat-sensitive hypothesis (Helfman 1989), natural selection should favor individuals that take action appropriate to the magnitude of threat, which would require an accurate predator recognition. Thus, recognition of and interest on predator odor by prey is of adaptive significance because it may reduce predation risk (Merkens et al. 1991; Amo et al. 2004b, 2005).

Lizards use their forked tongues to capture nearby molecules and deliver them to the Jacobson's organ which is part of the vomeronasal system (Halpern 1992). Given the sensory mechanism of the tongue and the ease of observing its protrusion, tongue flicking provides a quantifiable indication of an animal's chemosensory investigation of a stimulus source (Cooper and Burghardt 1990). According to this interpretation, increased tongue flicking (TF) rates would indicate an increased effort in detecting chemical signals and locating its source (Cooper 1994; Amo et al. 2004b). Thus, we used the TF rates of male lizards *I. monticola* in response to the scent of *C. austriaca* as a measure of interest of lizards on detecting the snake predator. We also measure TF rates to the scents of an inoffensive lizard species (*Podarcis muralis*), and to the scent of conspecific male and female *I. monticola*, as control measures of male general interest or intensity of chemosensory investigation of any scent. To determine males' chemical and visual attractiveness for females, we analyzed chemical composition of femoral gland secretions of males and performed female association trials to chemical or visual stimuli of males (López et al., 2002, 2003; Martín and López 2000, 2006; Olsson et al. 2003). If male *I. monticola* compensated behaviorally their chemical conspicuousness, we predicted that TF rates to the scent of the saurophagous snake *C. austriaca*, but not to the other scent stimuli, may correlate with chemical, but not visual, attractiveness of male lizards.

Methods

Study animals

Between February and April 2005, we captured by harmless noosing 20 recently post-hibernation emerging adult lizards *I. monticola* (10 males, SVL= 77 ± 2 mm; 10 females, SVL= 76 ± 4 mm) in moist and vegetated lowland habitat at coastal valleys in Galicia (43°70'N, 7°98'W; 100 m asl). We measured SVL and tail length of lizards to the nearest 1 mm with a ruler, and weigh to the nearest 0.01 g. Body condition was calculated from the residuals of the regression equation of log-body mass on log-SVL (Schulte-Hostedde et al. 2005). We also counted the number of shoulder blue ocelli on the right and left side (2–4 counts per animal), which are used as visual sexual signals. At least two months before testing, to allow acclimation to laboratory conditions and to homogenize previous recent predation risk experience, lizards were individually housed at "El Ventorrillo" Field Station (Navacerrada, Madrid prov.), in outdoor 60 x 40 cm PVC terraria containing rocks for cover.

We captured two smooth snakes, *Coronella austriaca*, in the same area, and four male wall lizards, *Podarcis muralis*, in the Guadarrama Mountains, few days before testing, which were housed in identical conditions than *I. monticola*. Snakes were not fed during their captivity conditions (less than two weeks). The photoperiod and ambient temperature were those of the surrounding region and water and mealworm larvae dusted with multivitamin powder were provided ad libitum for lizards. All lizards and snakes were healthy during the trials, and were returned to their exact capture sites at the end of experiments.

Analyses of femoral gland secretions

Immediately after capture in the field, we extracted femoral gland secretion of male *I. monticola* by pressing with forceps around the femoral pores. We analyzed lipophilic compounds in femoral secretions by using a ThermoQuest Trace 2000 gas chromatograph-mass spectrometer (GC-MS) fitted with a 30 m column (Supelco, Equity-5) (for details of similar analytical procedures see López et al. 2006). Initial identification of compounds was done by comparison of mass spectra in the NIST 1998 mass spectral library, and later confirmed with authentic standards. The relative amount of each component was determined as the percent of the total ion current (TIC). Areas of the peaks that represented >0.05% relative peak area (which together represent 96.8% of TIC area) were transformed following the formula of Aitchison (1986) (for similar analyses see Dietemann et al. 2003; López et al. 2006).

Female association trials

We performed female association trials to examined differences in female preferences for chemical or visual signals of different males. Female Iberian rock lizards use to avoid courtship by non-preferred males (López et al. 2003). Female association patterns to chemical or visual signals of a male (i.e., the time that a female spent on an area scent marked by a male or in proximity to a male; see below) may be used as a measure of female preference for that particular male (for similar procedures see López et al. 2002, 2003; Martín and López 2000, 2006; Olsson et al. 2003; Hamilton and Sullivan 2005).

For the chemical signals trials, we obtained the scents from male lizards by placing in each male's cage several absorbent paper strips (35x10 cm) fixed to the floor, and left them there for ten days. Papers were removed to be placed in females' cages immediately before each experiment. Each paper strip was used only in one test to avoid contamination.

For the visual signal trials, we placed two males, which were visually and chemically isolated between them, on two small cages (30x20 cm) contiguous to the female's cage. Females could see the two males through a plastic wall that allowed UV vision but that avoided perception of chemical stimuli.

Females' cages (60x40 cm) had a central refuge, two identical basking tiles at each side, and a water recipient. At the beginning of each test (07.00 hours GMT), when females were still inactive, we either fixed with fresh gloves on one tile one paper strip from a male, and another from a different male on the other tile (for chemical trials), or placed each male in the experimental cages covered with a small box during 5 min to acclimatized them before trials started (for visual trials). Each female was tested over 12 days, once a day, with six different pairs of males (or their papers) previously unknown. The males tested and the positions of the males' cages or papers were randomly determined.

Females were monitored each 10 min (between 1000 and 1400 h) from a hidden point, and their locations in the cages were recorded. These locations were considered to be representative of females' space use of the cages. If a female was located on either one of the two papers (chemical trials) or in front of one of one of the two males (visual trials), she was considered as having chosen temporally that particular male, whereas if she was located in a non specific position (e.g., central refuges), we assumed that she had made no choice. To ensure that females were exposed to both males and were aware of both male's stimuli, at least one recording in each male's section were considered necessary for a trial to be valid. This presumption was fulfilled in all tests. At the end of each trial, the papers or the males were removed and the cage was thoroughly rinsed with clean water to eliminate odors of precedent trials.

Males or different papers from each male were used in several choice tests (between 4 and 8) against other males or their papers, with different females. We calculated the average 'chemical attractiveness index' (Ch-AI) or 'visual attractiveness index' (Vi-AI) for each individual male from the sum of scores obtained in all his tests: the male section or the paper on which a female spent greater than 60% of her time (excluding time in the no choice area) was designed as the preferred stimuli in that trial, and we gave a score of 1 to the preferred male and 0 to the other male. When there were not such differences in time between the two males it was considered a tie, and we assigned a score of 0.5 to each male.

To analyze the relationships between Ch-AI and proportion of chemical compounds in femoral secretions we used stepwise general regression models (GRM) (using Statistica 6.0 software) with Ch-AI (after arcsine-root transformation to ensure normality) and the relative proportions of chemicals in femoral secretions of males (transformed TIC areas, see above) as potential predictors of the female preference. Data normality was verified by Shapiro-Wilk's tests, and tests of homogeneity of variances (Leveane's test) showed that variances were not significantly heterogeneous after transformation.

Tongue-flick measures of olfactory interest

We compared tongue-flick rates by male lizards *I. monticola* in response to stimuli arising from cotton swabs impregnated with the scents of (1) smooth snake (*C. austriaca*) (chemically-guided predator), (2) wall lizard (*P. muralis*) (lizard scent control), (3) deionized water (odorless control), (4) conspecific male *I. monticola* or (5) conspecific female *I. monticola*. Water was used to gauge baseline tongue-flick rates in the experimental situation. We prepared stimuli by dipping the cotton tip (1 cm) of a wooden applicator attached to a long stick (50 cm) in deionized water. The other stimuli were added by rolling the moistened cotton over the body surface of each stimuli donor. A new swab was used in each trial. Every lizard was exposed to each stimulus in counterbalanced order. One trial was conducted per day for each animal. We used groups of five (or four) males per session with each male in the group serving as test male and as donor of secretions to be presented to another of the five (or four) males. In this manner, we controlled for manipulation and collection of femoral secretions from test males before their use in the trials and were able to present stimuli to test males shortly after their preparation (thereby avoiding fading of stimuli). During the trials, male home terraria were devoid of refuges, water, and food bowls. Trials were conducted in outdoor conditions between 1100 and 1500 hours when lizards were fully active.

To begin a trial, the experimenter slowly approached the terrarium and slowly moved the cotton swab to a position 1 cm in front of the lizard's snout. The number of tongue-flicks directed to the swab was recorded for 60 s, beginning with the first tongue-flick. We recorded the time elapsed between presentation of the swab to a lizard and the first TF directed at the swab (latency period), and the numbers of TFs directed at the swab (directed TFs) and directed elsewhere (non-directed TFs) during 60 s counted from the first directed TF. Among stimuli differences in latency period and total TFs were analyzed using nonparametric statistics (Friedman's ANOVA by ranks and nonparametric *a pos-*

teriori multiple comparisons; Siegel and Castellan, 1988). The relationship between total TFs directed to any stimuli and chemical or visual 'attractiveness indexes' or proportion of chemical compounds in femoral secretions were analyzed using non-parametric statistics (Spearman's rank-order correlations) because the data were not normally distributed.

Results

Chemical signals and 'attractiveness indexes'

The lipophilic fraction of femoral gland secretions of individual male *I. monticola* used in this experiment contained as major components twelve steroids (95.50% of TIC on average) and five carboxylic acids (2.96%), but we also found two alcohols (1.15%), squalene (0.28%), and two lactones (=furanones) (0.11%). On average, the three most abundant chemicals were three steroids: cholesterol (79.93%), campesterol (7.56%), and sitosterol (2.07%). Dodecanoic acid (1.26%) was the most abundant fatty acid, and hexadecanol (1.05%) the most abundant alcohol.

'Chemical attractiveness indexes' (Ch-AI) of male *I. monticola* were significantly and positively correlated with the relative proportions of hexadecanol and negatively with a furanone (dihydro-5-tetradecyl-2(3H)-furanone) in their femoral secretions (forward stepwise GRM: R^2_{model} = 0.84, $F_{2,7}$ = 16.01, P = 0.002; Hexadecanol: β = 0.68, t = 4.21, P = 0.003; Furanone: β = -0.47, t = -2.92, P = 0.022). All other chemical compounds were not significantly related with Ch-AI and were not included in the final model. Thus male *I. monticola* with higher Ch-AI had femoral secretions with relatively higher proportions of hexadecanol and a furanone. Ch-AI and 'visual attractiveness indexes' (Vi-AI) were not significantly correlated (r_s = 0.12, N = 10, P = 0.73).

Tongue-flick measures of olfactory interest

All male *I. monticola* directed TFs to swabs in all conditions. There were significant differences among treatments in the latency period to the first TF (Friedman`s two-way ANOVA: χ^2_4 = 27.61, N = 10, P < 0.0001; Figure 1a). Thus, latencies to water or the scent of wall lizards were significantly higher than to any other stimuli (non-parametric post-hoc, P < 0.007 in all cases), but there were not significant dif-

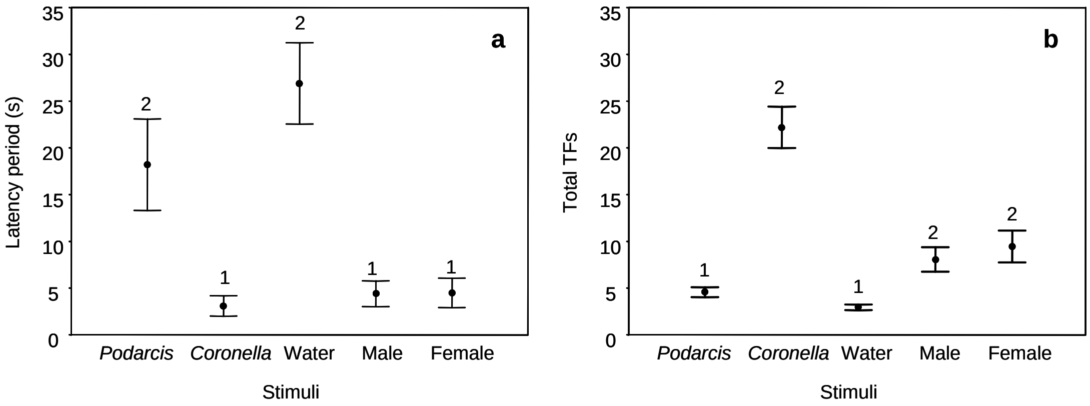

Figure 1. Mean (± SE) (a) latency period to the first TF and (b) total tongue-flicks directed by male lizards *I. monticola* to chemical stimuli from an innocuous lizard species (*Podarcis muralis*), a sympatric chemically-guided snake predator (*Coronella*), a baseline control (water), and conspecific male and female *I. monticola*. Same numbers above each stimulus mean no significant differences in post-hoc tests.

ferences in latency periods between water and the scent of wall lizards ($P = 0.26$), or between the scent of snake and the scent of male and female conspecific lizards ($P > 0.21$ in all cases).

There were significant differences among treatments in numbers of TFs (Friedman`s two-way ANOVA: $\chi^2_4 = 33.85$, $N = 10$, $P < 0.0001$; Figure 1b). Thus, responses of males to water were significantly lower than to all other stimuli (non-parametric post-hoc tests, $P = 0.005$), except for the scent of wall lizards, which approached significance ($P = 0.05$). Responses of male lizards to the scent of smooth snakes were significantly greater than to the scent of conspecific lizards and wall lizards ($P = 0.005$ in all cases), but responses to conspecific males and females were not significantly different between them ($P = 0.11$).

TFs directed to the scent of smooth snakes by male *I. monticola* were significantly and positively correlated with their respective Ch-AI ($r_s = 0.88$, $N = 10$, $P = 0.0005$; Figure 2a) and with the proportions of hexadecanol and negatively with a furanone in their femoral secretions (forward stepwise GRM: $R^2_{\text{model}} = 0.90$, $F_{2,7} = 34.88$, $P = 0.0002$; Hexadecanol: $\beta = 0.57$, $t = 4.90$, $P = 0.001$; Furanone: $\beta = -0.66$, $t = -5.72$, $P = 0.0007$; Figure 2b), but not with their Vi-AI, number of blue ocelli or body condition ($r_s < 0.32$, $P > 0.28$ in all cases). Chemosensory responses to water, wall lizards or conspecifics were not significantly correlated with Ch-AI, Vi-AI, blue ocelli, condition or with TFs directed to snakes ($r_s < 0.56$, $P > 0.09$ in all cases). Thus, olfactory interest of male lizards to the scent of snakes was only related to their own chemical attractiveness for conspecific females (both Ch-IA and chemical compounds in secretions) but not with their visual attractiveness (both Vi-AI and number of blue ocelli). Moreover, only responses (TFs directed) to the scent of snakes, but not to other stimuli, were correlated with chemical attractiveness.

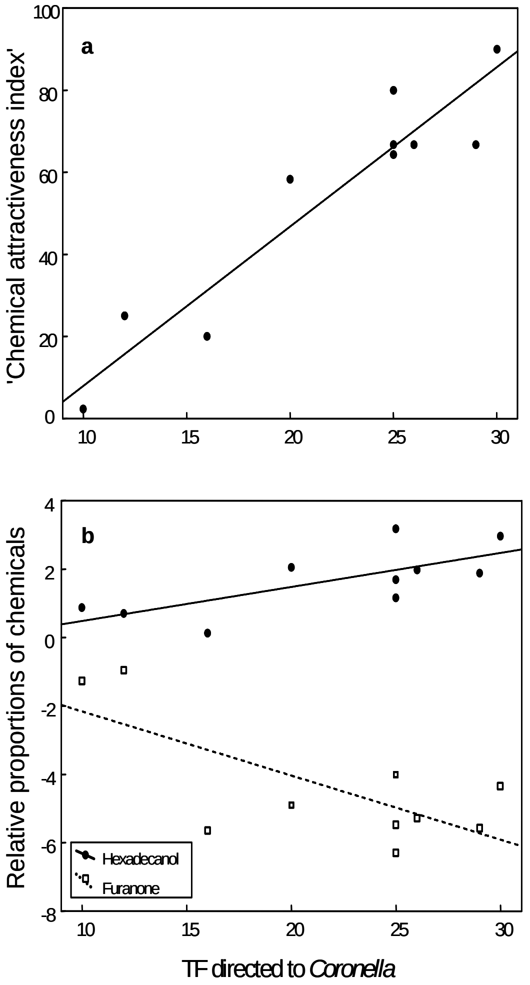

Figure 2. Relationships between the number of total TFs that male *I. monticola* directed to chemical stimuli from a chemically-guided snake predator (*Coronella*) and a) the 'chemical attractiveness indexes' or b) the relative proportions (transformed TIC areas) of hexadecanol and dihydro-5-tetradecyl-2(3H)-furanone in the femoral secretions of those males.

Discussion

Our results showed that individual male lizards *I. monticola* with higher 'chemical sex appeal' for females also showed more interest on the scent of their chemically-guided snake predators. This suggests interindividual differences in antipredatory strategies of male lizards mediated by their own chemical conspicuousness, thus compensating for an increased predation risk.

Animals balance increased risk of predation with the opportunity to perform several reproductively important behaviors (Cooper 1999). An important component of antipredator behavior is the ability to detect and recognize predators (Lima and Dill 1990). We found that male *I. monticola* are able to discriminate between scents from the predatory snake and other harmless lizard species or conspecifics. Differential tongue-flick rates and shorter latencies suggest that scents of smooth snakes are quickly recognized and elicited a higher chemosensory interest than other scents. Many antipredator adaptations are induced or mediated by the ability of the prey to recognize chemical cues from the predator (Kats and Dill 1998) and show behavioral changes and specific defensive responses (Van Damme et al. 1990; López and Martín 2001; Cabido et al. 2004). These changes of behavior should aid prey to avoid being captured by the predator, so a higher responsiveness to predator scents may increase prey survival probabilities (Downes 2002). According to the threat-sensitive hypothesis (Helfman 1989), natural selection should favor individuals that take action appropriate to the magnitude of threat, which typically has been suggested to depend on predator characteristics or threat level (e.g., Amo et al. 2004b; Cabido et al. 2004). However, predation threat may also depend on interindividual variations in prey vulnerability. For example, Stapley and Keogh (2004) showed that antipredatory behaviors differed between territorial and non-territorial male lizards, which may be due to the different predatory risk suffered by each one due to their different activity rates. Amo et al. (2004c) also provided evidence that chemosensory discrimination by lizards of snake chemicals is thermally dependent, and, thus, suboptimal body temperatures may impede a lizard's ability to avoid snake-scented refuges or modified their refuge-use strategies (Martín and López 1999a; Amo et al. 2004c, 2005). Similarly, an increased chemical conspicuousness of a male lizard, which may be advantageous in terms of sexual selection, may also increase the risk of been preyed by chemically-guided predators (Zuk and Kolluru 1998; Koivula and Korpimäki 2001; Rossell and Sanda 2006) and should be behaviorally compensated. In accordance, we found that olfactory interest of male *I. monticola* for the scent of smooth snakes was correlated with their own chemical attractiveness to females and with the compounds of their chemical signals that were related to attractiveness. This suggests that more chemically conspicuous males may have different antipredatory strategies. For instance, they may pay more attention to predator cues to then behave more shy (López et al. 2005; Cabido et al. 2009), avoid areas occupied by those predators (Amo et al. 2005) or vary their refuge use (Martín and López 1999a). Attractiveness to females may mirror conspicuousness to specialized predators because predators usually exploit sexual signals to locate prey (Zuk and Kolluru 1998; Ryan et al. 1982).

Moreover, we found that the relative proportions of chemical compounds in secretions of males that were related with attractiveness to females also correlated with the male interest on smooth snake cues. Thus, male lizards with more hexadecanol and less furanone in their femoral secretions were more attractive to females, showed higher dominance status (Martín et al. 2007), and were also those more interested on the snake cues. Although it has been shown that smooth snakes use lizard chemical signals to locate and prey on them (Amo et al. 2004b), future studies are needed to determine whether smooth snakes actually use the same chemicals related to attractiveness to locate and prey on this lizard, and whether snakes can use other chemicals to locate and prey on other lizard species having different chemical compounds related to male attractiveness or dominance (Martín and López 2006; Cabido et al. unpublished data).

Attractiveness of male lizards due to visual signals, which was not related with chemically attractiveness, or number of blue ocelli, which are related with shyness towards visual predators (Cabido et al. 2009), were not related with male olfactory interest on scent of smooth snake. Therefore, only chemical conspicuousness, but not visual one, correlates with response to chemically-guided predator cues. Body condition, which may influence antipredatory behavior (Martín 1996; Martín and López 1999a; Cabido et al. 2009), neither was related to responses to smooth snake cues. Finally, only responses to scent of snakes, but not to other stimuli, were correlated with chemical attractiveness. That is, males with more attractive chemical signals did not respond more to any chemical stimuli due to an enhanced reproductive state or a higher reproductive inversion (Huntingford 1976; Riechert and Hedrick 1993; Dall et al. 2004).

Most studies show that visual or acoustical conspicuousness correlates positively with shyness (Forsman and Appelqvist 1998; Hedrick 2000; Cuadrado et al. 2001; Lindström et al. 2007; Cabido et al. 2009; but see Godin and Dugatkin 1996) and similar phenomena may occur with chemical signaling. Although the increased risk of predation due to chemical conspicuousness is usually ignored as a cost of sexual selection (Greenfield 1981; Boake et al. 1996; Wolff 2004), predation risk has recently been suggested as a powerful agent of counter selection on olfactory signaling behavior (Rosell and Sanda 2006). However, behavioral compensation of chemical conspicuousness may allow animals to override the increased risk of predation by chemically-guided predators. Behavioral compensation may be an extended behavioral mechanism that explains the lack of scent marking decrease by voles in response to the odor of a predator found in some laboratory and field studies (Wolff 2004). Behavioral compensation may also explain that dominant male mice (*Mus musculus*) reduce scent marking in the presence of predator cues, whereas subordinate mice do not (Roberts et al. 2001). Chemical signals of dominant males might be more conspicuous than subordinates' ones, so behavioral compensation for predation may be only necessary in the former.

On conclusion, behavioral compensation of chemical conspicuousness may explain the persistence of conspicuous chemical signals or scent marking in species that suffer high predation risk. A recent study (Oufiero and Garland 2007) illustrates the perils of ignoring potential compensatory mechanisms when studying the costs of sexually selected traits. It was long ago suggested that by altering behavioral patterns in the face of new environmental conditions, organisms can minimize exposure to new selective pressures and retard evolutionary change (Wake et al. 1983; but see Losos et al. 2004). However, most studies on chemical mediated predation ignore potential compensatory antipredatory behavior, which may lead to erroneous conclusions about the cost of chemical signals.

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Capítulo 4

El Sistema de Apareamiento Puede Predecir las Preferencias de las Hembras por Diferentes Rasgos de las Señales Masculinas en Dos Especies Hermanas

Las hembras a menudo prefieren los rasgos de los machos que están correlacionados con la dominancia, aunque otras veces ocurre lo contrario. Cuando los rasgos de las señales seleccionados por la competencia entre machos no reflejan completamente su calidad, se espera que las hembras utilicen otras señales para seleccionarlos. En este trabajo hemos planteado que diferentes sistemas de apareamiento pueden determinar los beneficios directos y/o indirectos que los machos proveen a sus parejas y, por tanto, sesgar las preferencias de las hembras por diferentes rasgos de las señales de los machos. Así, en las especies más territoriales, las hembras deberían preferir rasgos relacionados con la dominancia, mientras que en las especies menos territoriales las hembras deberían preferir otros rasgos de las señales de los machos relacionadas también con su calidad genética heredable. Hemos examinado nuestras predicciones en dos especies de lagartijas estrechamente relacionadas con diferentes condiciones ecológicas que habrían promovido diferentes sistemas de apareamiento. Para estimar diferencias entre especies a nivel de intensidad competitiva y sistema de apareamiento, hemos medido el dimorfismo sexual en el tamaño de la cabeza, que refleja la competencia intrasexual, y el microhábitat seleccionado *vs.* el disponible, que refleja la distribución espacial. Para examinar las diferencias entre especies en cuanto a las preferencias de las hembras por los rasgos de las señales de los machos, determinamos la respuesta inmune de éstos y la composición química de sus secreciones femorales, para comparar, dentro de cada especie, los patrones de asociación de las hembras con los rasgos químicos de los machos relacionados con la dominancia o con la respuesta inmune. Nuestros resultados mostraron diferencias entre especies en el sistema de apareamiento y en las preferencias de las hembras por los rasgos de las señales de los machos. En la especie más territorial los rasgos de las señales relacionados con la dominancia son preferidos por las hembras, mientras que los rasgos de las señales relacionados con la respuesta inmune son preferidos en la especie menos territorial. Ambas especies prefirieron señales de los machos con mejor respuesta inmune, que sugiere que diferentes mecanismos pueden estar involucrados para asegurar la honestidad de las señales en cada especie ■

MATING SYSTEM MAY PREDICT FEMALE PREFERENCES FOR DIFFERENT MALE SIGNAL TRAITS IN TWO SISTER LIZARD SPECIES

Females may often prefer male traits that are correlated with dominance, but other times the opposite is found. When signal traits selected by male-male competition do not reflect overall mate quality, females are expected to use other choice cues. We hypothesized that different mating systems may determine the direct and/or indirect benefits that males provide to their mates and, therefore, drive female preferences for different male signal traits. Thus, we predicted that in more territorial species females may prefer traits related to dominance, whereas in less territorial species, females may prefer other traits of male signals related to heritable genetic quality. We examined our prediction in two closed related lizard species with different ecological conditions, which may have promoted different mating systems. To estimate between-species differences in the level of competitive intensity and mating system, we measured head sexual dimorphism, which reflects intrasexual competition, and selected *vs.* available microhabitat, which reflects spatial distribution. To examine between-species differences in female preferences for male signal traits, we determined male immune response and chemical composition of male femoral gland secretions, and compared, within each species, the patterns of female association with male chemical traits related to dominance or to immune response. Our results showed between-species differences in the mating system and in female preferences for male signal traits. In the more territorial species, signal traits related to dominance are preferred, whereas signal traits related to immune response are preferred in the species less territorial. Both species preferred signals of males with better immune response, which suggest that different mechanisms may be involved to ensure honesty in each species.

Mate choice may lead either to the production of offspring with genotypes that increase viability ('good genes' or 'viability genes'), and/or to the production of sons with genotypes that make them more attractive (Fisherian traits) (reviewed in Kirkpatrick and Ryan 1991; Andersson 1994). Although the classical sexual selection models (Fisher process, good genes and/or handicap and/or indirect benefits and direct benefits), explore how signal content is established or maintained (Kirkpatrick and Ryan 1991; Andersson 1994), the evolutionary causes of divergence among closed related species in signal information content are scarcely reported. Different mate-choice tactics may exist in different species when males display multiple desirable features that confer different benefits to females under variable environmental conditions (Endler and Houde 1995). For example, females may often prefer male traits that are correlated with dominance, but other times the opposite is found (reviewed in Qvarnström and Forsgren 1998; Wong and Candolin 2005).

Characters selected by male contests are expected to be important cues also in female choice because they may signal quality honestly (Berglund et al. 1996). The armament–ornament model (Berglund et al. 1996; reviewed in Wong and Candolin 2005) predicts that females are expected to “exploit” signal traits used in male–male contests because the reliability of these traits is constantly put on trial in these contests and cannot be faked without incurring high costs. Indeed, female preference for dominant males has been found in many species (e.g., Borgia and Coleman 2000; Tarof et al. 2005).

However, although mating success tends to be skewed in favour of dominant males, an increasing number of studies are showing that dominant males are not always preferred by females (Moore and Moore 1999; López et al. 2002; Andersson et al. 2002). When signal traits selected by male-male competition do not reflect overall mate quality, females are expected to use other choice cues and might occasionally prefer subordinate males (Qvarnström and Forsgren 1998; Wong and Candolin 2005). Although this topic has been hypothesized, it has never been explored.

In this context, we hypothesized that different mating systems may determine the direct and/or indirect benefits that males can provide to their mates and, therefore, drive female preferences for either traits related to dominance or other traits of male signals related to heritable genetic quality. These differences in female benefits may lead to the divergence of the male signal information content through female mate choice.

The Cantabrian rock lizard (*Iberolacerta monticola*) and the Carpetane rock lizard (*Iberolacerta cyreni*) are two closely related and recently isolated species (Carranza et al. 2004; Martín 2005a,b) which may be adequate to explore this topic. Chemical signalling is well-developed in both species, where scent marks from femoral gland secretions of males could convey information about social status (Aragón et al. 2001; Moreira et al. 2006; Martín and López 2007; Martín et al. 2007) or about other traits that indicate presumably heritable genetic quality, such as immune response (Martín and López 2000a, 2006a,b; López et al. 2003). Male *I. monticola* of higher dominance status produce chemical signals with higher proportions of hexadecanol and octadecanol while controlling for male body size (Martín et al. 2007; Moreira et al. 2006). On contrast, proportions of cholesterol in femoral gland secretions may reliably signal fighting ability in male *I. cyreni* (Martín and López 2007). Moreover, male *I. cyreni* with a greater T-cell immune response had higher proportions of ergosterol and dehydrocholesterol in their femoral secretions (Martín and López 2006a).

Both species select microhabitats with high cover of large rocks with crevices that provide refuges (Martín and Salvador 1997; see results) but there are between-species differences in habitat structure (see results) which are expected to result in different mating systems. Variation in mating systems, when males do not provide parental care, arises because of differences in the spatial or temporal distribution of resources (food, suitable temperatures, refuges), which will influence how females are distributed and, hence, the ability of males to monopolize more females than other males (Emlen and Oring 1977; Arnold and Duvall 1994). A change in the distribution of females and males is likely to affect the nature and intensity of sexual selection (Arnold and Duvall 1994) and then, affect female mating preferences. Thus, when spatial distribution of females allows some males to achieve greater than average fitness, signalling dominance could be advantageous (Andersson 1994; Olsson 1994) and females may evolve preference for male traits that signal dominance (Qvarnström and Forsgren 1998). In this situation, females that mate with dominant males may gain direct benefits (e.g., Candolin and Voigt 2001) or indirect benefits if competitive ability is heritable and their sons enjoy similar competitive ability (e.g., Moore 1990; Alatalo et al. 1991; but see Qvarnström and

Forsgren 1998). In contrast, if females are distributed unpredictably, males will have to search extensively for mates, there will be a lack of male territoriality (e.g., Bondrup-Nielsen and Ims 1990), and females may not gain benefit if mate with dominant males (Wong and Candolin 2005; Qvarnström and Forsgren 1998). Moreover, when dominant males impose direct costs on females, such as increased risk of injury (Le Boeuf and Mesnick 1991; Fitze et al. 2008) females may prefer traits unrelated to dominance or discriminate against dominant males (Qvarnström and Forsgren 1998).

Both species of Iberian rock lizards also differ in mating season duration (*I. cyreni*: June-July; *I. monticola*: April-July) (Martín 2005a,b; Rúa and Galán 2003) and *I. monticola* present lower densities (52.5-150 ind/ha), larger male home ranges and lower male home range overlap (Galán unpublished data; Moreira et al. 1998), than *I. cyreni* (220-328 ind/ha) (Martín and Salvador 1997; Aragón et al. 2004).

Therefore, space distribution, mating season duration and density differences may promote that *I. monticola* have a more territorial mating system, with higher male-male competition, than *I. cyreni*. In this context, we expected that male dominance may provide different benefits in each species and female preferences for different signal traits (signalling or not dominance) may diverge between both species of Iberian rock lizards. Thus we predict that female *I. monticola* may prefer male traits related to dominance, whereas female *I. cyreni* may prefer other traits of male signals related to heritable genetic quality.

To test this prediction, we examined between-species divergence in spatial distribution and in female mate preferences. To estimate between-species differences in mating system, we measured head sexual dimorphism, which reflects male competition (Gvozdík & VanDamme, 2003), and selected *vs*. available microhabitat, which reflects spatial distribution. In lizards, "space distribution" is mediated by refuge availability (Van Damme et al. 1989: Lemos-Espinal and Ballinger 1995; Martín and Salvador 1997) and thermoregulation requirements (Sorci et al. 1996; Bashey and Dunham 1997). Large male lizards may defend territories (only during the reproductive season) in areas that are micro-climatically more suitable for females than surrounding areas (Wikelski and Trillmich 1997). Thus, refuge and thermoregulatory appropriate habitats should predict male and female spatial distribution in space and time and may have promoted different social scenarios. Finally, to estimate between-species differences in female mate preferences, we determined male immune response and the chemical composition of male femoral gland secretions and compared, within each species, the patterns of female association with scent marks of males to explore what type of signal traits (i.e. those signalling dominance or health) are preferred by females.

Methods

Study specimens

Iberian rock lizards are small diurnal lacertid lizards found mainly in rocky habitats. Recent molecular studies and taxonomic revisions suggest that Iberian rock lizards group ("*Iberolacerta monticola*") comprise several closely related species and subspecies that occupy different isolated high mountain and some sea level areas in the Iberian Peninsula (Carranza et al. 2004). The Carpetane rock lizard, *Iberolacerta cyreni* (formerly *Lacerta monticola cyreni*) is found at high altitude mountains in Central Spain, occupying a sparsely vegetated habitat (Martín 2005a) whereas the Iberian rock lizard *Iberolacerta monticola* (formerly *L. monticola cantabrica*) is found in lower mountains and lowland valleys, even at the sea level, in Northwestern Spain, occupying a moist, more vegetated habitat (Galán and Fernández-Arias 1993; Galán 2008).

During February 2005, we captured by harmless noosing 16 recently post-hibernation emerging adult male lizards *I. cyreni* (snout-to-vent length, SVL, mean ± SD = 77 ± 3 mm) and 10 females (78 ± 4 mm) in alpine habitat at the Guadarrama Mountains in Madrid (40°77'N, 4°1'W; 1950 m asl), and 17 adult male lizards *I. monticola* (77 ± 2 mm) and 10 females (76 ± 4 mm) in more moist and vegetated lowland habitat at coastal valleys in Galicia (43°70'N, 7°98'W; 100 m asl). We calculated the relative head measures as the residuals from the regression equations of head measures on SVL (both log transformed). We performed general linear models (GLM) with the relative head measures to compare sexual head dimorphism between both species.

Only adult lizards of similar body size/age and with intact or fully regenerated tails were considered. Within each locality, we captured lizards in different places over large areas (2 km^2) to ensure that individuals had not been in previous contact, which might affect their responses (López and Martín 2002a). Males of both species were individually housed at "El Ventorrillo" Field Station (Madrid) in outdoor 60 x 40 cm PVC terraria containing rocks for cover. Water and mealworm larvae dusted with multivitamin powder were provided *ad libitum*. Before testing, we assessed female reproductive status by palpation of oviducts and checking male reproductive status observing productive status of femoral glands secretions.

Microhabitat characterization

We searched for lizards by walking during April between 07:00 and 14:00h (GMT) with consistent sampling effort in the two study localities. We recorded microhabitat data at the point where each individual was first sighted (*I. cyreni*: n = 43; *I. monticola*: n = 28). Four 1 m transects were laid out radiating from this point along the four cardinal directions, and records made at 5, 10, 15, 25, 75 and 100 cm. We noted the presence at substrate level of grass, leaf litter, bare sandy soil, small rocks (< 25 cm), medium rocks (25-100 cm), large rocks (100-200 cm), or rocky outcrops and cliff walls (> 200 cm). Plant contacts at 5, 10, 25 and 50 cm height were also noted, using a calibrated stick, for grasses or small herbaceous semi-perennial plants (<50 cm height), large woody bushes (*Cytisus oromediterraneus* and *Juniperus comunnis* in Guadarrama Mountains, or *Cytisus scoparius* and *Ulex europaeus* in Galicia) or trees (*Pinus sylvestris*). We also noted whether the sample point was in a sunny or shaded location, the presence of canopy tree cover above each point, and the distance to the nearest potential refuge (rock crevice or protective vegetation cover). This procedure provided 24 sample points per lizard location and allowed us to calculate the percent cover values of each habitat variable (i.e., % contacts with each substratum, vegetation type and sunny spots, and the mean distance to the nearest refuge; for a similar sampling methodology see Martín and Salvador 1997). To estimate the availability of microhabitats in a large area surrounding that actually used by lizards, we used a similar procedure to record the same variables as described above at 2, 3 and 4 m along the four cardinal directions from each sample point.

We used a principal component analysis (PCA) to reduce the microhabitat variables to a smaller number of independent components. Only principal components (PCs) with eigenvalues that explained more than 5% of the total variation were retained for further analysis. Original data (% contacts) were normalized by means of arcsine-square root transformation. Because of the large number of "zero" observations for many variables, some transformed data were not normally distributed, but otherwise met the assumptions of a PCA. Deviations from normality do not necessarily bias the analysis, as long as the distributions are reasonably unskewed (Legendre and Legendre 1998). Thus, we considered that our transformations reduced the skew sufficiently to minimize the risk of bias in the analysis. We used general linear models (GLM) to compare PC scores describing microhabitat characteristics in relation to each species (*I. monticola* vs. *I. cyreni*), sex and the type of point (available vs. used by lizards; within factor) to determine whether lizards used available microhabitats in a non-random fashion. We included the interaction between species, sex and type of point in the model to test for differences in microhabitat selection of lizards within each sex and species. Post-hoc pairwise comparisons were planned using Tukey's honestly significant difference tests (Sokal and Rohlf 1995).

Measurement of immune response

We used the phytohaemagglutinin (PHA) injection test (a delayed-type hypersensivity test) to assess the T cell-mediated immune (CMI) response in vivo (Smits et al. 1999; Belliure et al. 2004). We measured with a pressure-sensitive spessimeter the thickness (to the nearest 0.01 mm) of the right hindlimb foot pad at a marked point before and after 24 h of injecting 0.02 mg of PHA dissolved in 0.01 ml phosphate-buffered saline at this point. We calculated the CMI response as the difference between pre- and postinjection thickness measures (Smits et al. 1999). The only appreciable effect of the PHA injection was a slight swelling of the skin, which disappeared after 48 h. No lizard showed any sign of stress or pain in these tests or during the trials. Lizards maintained good conditions throughout the study and were returned to their capture sites at the end of trials.

Analyses of femoral gland secretions

Immediately after capture in the field, we extracted femoral gland secretion of males by pressing with forceps around the femoral pores. We analyzed lipophilic compounds in femoral secretions by using a ThermoQuest Trace 2000 gas chromatograph-mass spectrometer (GC-MS) fitted with a 30 m column (Supelco, Equity-5) (for details of the analytical procedures see López et al. 2006). Initial identification of compounds was done by comparison of mass spectra in the NIST 1998 mass spectral library, and later confirmed with authentic standards. The relative amount of each component was determined as the percent of the total ion current (TIC). Areas of the peaks that represented >0.05% relative peak area (which together represent 96.8% of TIC area) were transformed following the formula of Aitchison (1986) and used as variables in a two separated principal component analysis (PCA) for each species (for similar analyses see Dietemann et al. 2003; López et al. 2006). The PCA for relative proportions of chemical compounds in secretions of male *I. monticola* produced six components that together accounted for the 87.5 % of the variance, and the PCA for chemicals of male *I. cyreni* produced seven components that together accounted for the 91.0 % of the variance. The principal components (PC) extracted for each species were used as independent variables in within species analyses (see below).

Female association trials

We perform female association trials within each species to examined differences in female preferences for male chemical signals. Female Iberian rock lizards avoid courtship by non-preferred males (López et al. 2003), so female association patterns to male chemical signals may indicate female preferences and it has been used as a measure of female preferences in several studies (López et al. 2002, 2003; Martín and López 2000a, 2006a; Olsson et al. 2003). We used the time that females spent in the scents of males as a measure of her preference for that male (for a similar procedure see Martín and López 2000a, 2006a; López et al. 2003; Olsson et al. 2003). To obtain the scents from male lizards, we had placed in each cage several absorbent paper strips (35x10 cm) fixed to the floor, and left them there for ten days. Papers were removed to be placed in females' cages immediately before each experiment. Females' cages (60x40 cm) had a central refuge, two identical basking tiles at each side, and a water recipient. At the beginning of each test (07.00 hours GMT), when females were still inactive, we fixed with fresh gloves on one tile one paper strip from a male, and another from a different male on the other tile. Each female was tested over 6 days, once a day, with papers from two different pairs of previously unknown males. The males tested and the positions of papers were randomly determined. Each paper strip was used only in one test to avoid contamination.

Females were monitored each 10 min (between 1000 and 1400 h) from a hidden point, and their locations in the cages were recorded. These locations were considered to be representative of females' space use of the cages. If a female was located on either of the two papers, she was considered as having chosen temporally that particular paper, whereas if she was located in

Table 1 Major lipophilic compounds found in femoral gland secretions of individual male lizards *I. cyreni* and *I. monticola* used in this study. The relative amount of each component was determined as the percent of the total ion current (TIC) and reported as the average (± 1SE).

Compound	*I. cyreni*	*I. monticola*
Steroids:		
Cholesterol	88.92 ± 0.92	79.93 ± 1.00
Ergosta-5,22-dien-3-ol	0.04 ± 0.03	-
Cholestan-3-one	-	0.99 ± 0.09
Ergosterol	0.10 ± 0.07	0.96 ± 0.15
Stigmasta-5,24(28)-dien-3-ol	1.95 ± 0.83	0.95 ± 0.26
Campesterol	4.13 ± 0.58	7.56 ± 0.46
4-Methyl-cholest-7-en-3-ol	0.01 ± 0.01	0.18 ± 0.06
Ergosta-5,8-dien-3-ol	0.04 ± 0.03	0.13 ± 0.04
Unidentified steroid	-	0.85 ± 0.15
γ-Sitosterol	1.88 ± 0.21	2.07 ± 0.15
24-Propylidene-cholest-5-en-3-ol	0.15 ± 0.09	0.09 ± 0.03
4,4-Dimethyl-cholest-7-en-3-ol	0.06 ± 0.05	0.47 ± 0.12
4,4-Dimethyl-cholesta-5,7-dien-3-ol	0.23 ± 0.14	1.32 ± 0.21
Carboxylic acids and their esters*:*		
Dodecanoic acid	1.27 ± 0.24	1.26 ± 0.23
Hexadecanoic acid	0.43 ± 0.08	0.96 ± 0.16
Octadecanoic acid	0.06 ± 0.02	0.16 ± 0.04
Octadecenoic acid	0.11 ± 0.04	0.49 ± 0.09
Octadecadienoic acid	0.07 ± 0.03	0.10 ± 0.03
Alcohols:		
Hexadecanol	-	1.09 ± 0.13
Octadecanol	0.01 ± 0.01	0.11 ± 0.03
Others:		
Squalene	0.54 ± 0.12	0.28 ± 0.05
4-Hydroxy-hexadecanoic acid γ-lactone	0.03 ± 0.02	0.09 ± 0.03
4-Hydroxy-octadecanoic acid γ-lactone	-	0.02 ± 0.01

a non specific position (e.g., central refuges), we assumed that she had made no choice. To ensure that females were exposed to both males' tiles and were aware of both male's stimuli, at least one recording in each male's section were considered necessary for a trial to be valid. This presumption was fulfilled in all tests. At the end of each trial, the papers were removed and the cage was thoroughly rinsed with clean water to eliminate odours of precedent trials.

Different papers from each male were used in several choice tests (between 4 and 8) against the papers of other males, with different females. We then calculated the relative time that a female spent on the paper scent marked by each individual male (time on paper of each male / time on papers of both males). We also calculated the average 'attractiveness index' for each individual male from the scores obtained in all his tests: the paper on which a female spent greater than 60% of her time (excluding time in the no choice area) was designed as the preferred paper in that trial, and we gave a score of 1 to the preferred male and 0 to the other male. When there were not such differences in time between the two papers it was considered a tie, and we assigned a score of 0.5 to each male.

To examine species differences in female preferences for characteristics of males chemical signals, we used, within each species, general regression models (GRM) (using Statistica 6.0 software) with both, the relative time that females spent on the papers scent marked by males and male 'attractiveness index' (log-transformed), and the principal components (PC) resulting from the PCA for relative proportions of chemicals in femoral secretions of males (see above) as potential predictors of the female preference. Data normality was verified by Shapiro-Wilk's tests, and tests of homogeneity of variances (Leveane's test) showed that variances were not significantly heterogeneous after transformation. To decide what variables best predicted female preference, we used a "best-subsets" approach in conjunction with stepwise methods (Neter et al. 1985). We chose the most parsimonious model as having the lowest Akaïke information criterion (AIC), and we checked its coincidence with the equivalent stepwise solution. Residuals from the final models were normally distributed (Grafen and Hails 2002). Finally, we used linear regressions to assess within each species the relationships between the immune response of males (log-transformed) and female preferences (relative time or attractiveness index).

Results

Sexual dimorphism

Relative head length was significantly greater in males than in females in the two lizard species ($F_{1,64}$ = 572.3, P < 0.0001; adjusted model, R^2 = 0.89, $F_{3,64}$ = 197.6, P < 0.0001) but the degree of sexual dimorphism in head length was greater in *I. monticola* than in *I. cyreni* (interaction: $F_{1,64}$ = 5.2, P = 0.02). Relative head width and height were also significantly greater in males than in females in both species ($F_{1,64}$ > 205.6, P < 0.0001 in both cases; adjusted model R^2 > 0.80, $F_{3,64}$ > 93.8, P < 0.0001), but the degree of sexual dimorphism in head width and height did not significantly differ between species (interaction: $F_{1,64}$ < 2.7, P > 0.1 in both cases).

Microhabitat use

The PCA for microhabitats available and those used by lizards produced six components that together accounted for the 74.84 % of the variance (Supplement 1). There were significant differences in relation to all PCs between the two lizard spe-

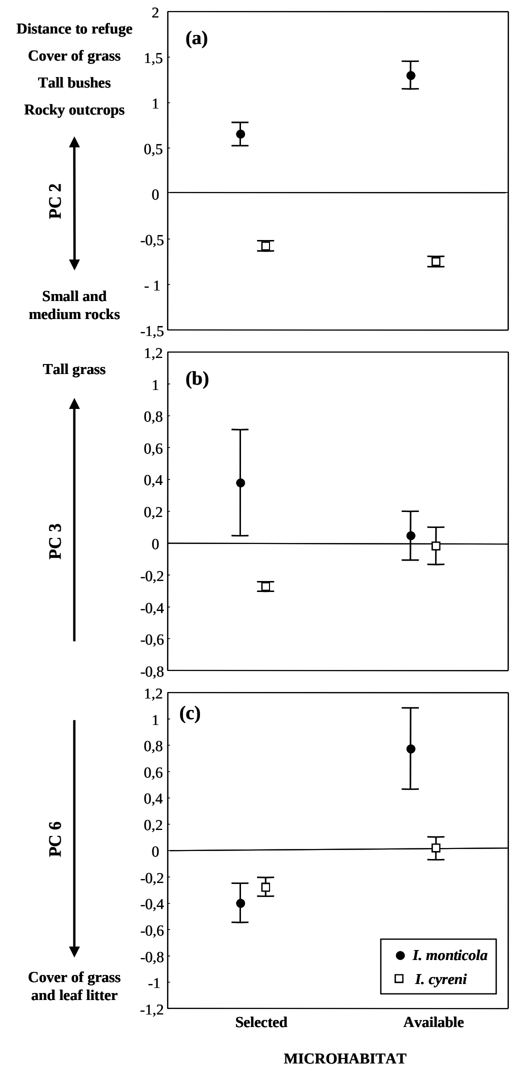


Fig. 1 Mean (± SE) PC scores (a: PC-2; b: PC-3 and c: PC-6) for microhabitat used and available by adult *I. monticola* lizards (black circles) and adult *I. cyreni* lizards (open circles).

cies (GLM, Wilks' $\lambda = 0.22$, $F_{6,131} = 76.54$, $P < 0.0001$) and between types of microhabitat points (available *vs.* used by lizards; Wilks' $\lambda = 0.70$, $F_{6,131} = 9.15$, $P < 0.0001$) but interaction between sex and species, sex and type of point or between sex, species and type of point were not significant ($P > 0.07$ in all cases). The interaction between species and type of point was significant (Wilks' $\lambda = 0.82$, $F_{6,131} = 4.56$, $P < 0.0001$). The general model showed significant overall differences between species for PC-2 (adjusted $R^2 = 0.70$, $F_{7,136} = 50.74$, $P < 0.0001$), PC-4 (adjusted $R^2 = 0.09$, $F_{7,136} = 3.09$, $P = 0.004$) and PC-6 (adjusted $R^2 = 0.18$, $F_{7,136} = 5.52$, $P < 0.0001$), but not for the other PCs (adjusted $R^2 < 0.08$, $F_{7,136} < 1.79$, $P > 0.09$ in all cases).

There were significant differences between species in characteristics of available microhabitats defined by PC-2 (Tukey's tests, $P < 0.0001$) and PC-6 scores ($P = 0.003$). Thus, in the habitat of *I. cyreni* there was a higher cover of small and medium rocks, with many crevices, and refuges were closer, whereas in the habitat of *I. monticola* there was more cover of grass, tall bushes and rocky outcrops (PC-2; Figure 1a) and more cover of grass and leaf litter (PC-6; Figure 1c). Similarly, there were significant differences between species in the PC-2 ($P < 0.0001$) and PC-3 ($P = 0.03$) scores of microhabitat used by lizards. Thus, *I. cyreni* used microhabitats with higher cover of small and medium rocks, many crevices, and closer to refuges, whereas *I. monticola* used microhabitats with higher cover of rocky outcrops, small plants and grass (PC-2; Figure 1a), and with taller grass (PC-3; Figure 1b). Therefore, microhabitats available and selected by both lizard species differed in refuge availability or distance to the nearest refuge.

There were not significant differences between microhabitats available and used by male *I. cyreni* in all PC scores (Tukey's tests, $P > 0.29$ in all cases), but there were significant differences between microhabitat available and used by male *I. monticola* in PC-2 and PC-6 scores ($P < 0.0001$ in both cases). Thus, microhabitat availability and microhabitat preferences corresponded in *I. cyreni*, whereas *I. monticola* selected microhabitats with less cover of tall woody bushes, more small and medium rocks and closer to refuges (PC-2; Figure 1a) and with less cover of grass or leaf litter (PC-6; Figure 1c) than those available in their habitat.

Chemical composition of males' femoral gland secretions

The lipophilic fraction of femoral gland secretions of individual male *I. cyreni* and *I. monticola* used in this experiment were similar in that both species had as major components steroids (96.5% of TIC on average) and carboxylic acids (3.4 %), but we also found alcohols, squalene and lactones (Table 1). On average, the three most abundant chemicals were cholesterol (84.4%), campesterol (5.8%), and sitosterol (2.0%). However, there were some interspecific clear differences in the presence/absence of five major chemicals.

Exclusive compounds of male *I. monticola* were cholestan-3-one and an unidentified steroid, a lactone, and especially hexadecanol, which was found in appreciable quantities (1.1%) in this species, while ergosta-5,22-dien-3-ol was only found in *I. cyreni* (Table 1). Moreover, the comparison of relative proportions of the 18 shared compounds (transformed TIC areas) showed significant differences between species (MANOVA, Wilks' $\lambda = 0.048$, $F_{18,21} = 23.31$, $P < 0.0001$), which were explained because *I. cyreni* had significantly higher proportions of cholesterol (protected one-way ANOVA with significance level adjusted for multiple comparisons, $F_{1,38} = 66.82$, $P < 0.0001$) and squalene ($F_{1,38} = 9.29$, $P = 0.004$), and significantly lower proportions of ergosterol ($F_{1,38} = 11.54$, $P = 0.002$) and 4,4-dimethyl-cholesta-5,7-dien-3-ol ($F_{1,38} = 14.78$, $P < 0.0005$) than *I. monticola*.

Female association trials

'Attractiveness indexes' (AI) of male *I. monticola* were significantly and positively correlated with PC-1 resulting from the PCA for relative proportions of chemicals in their femoral secretions ($R^2_{\text{model}} = 0.54$, $F_{4,12} = 3.96$, $P = 0.028$; PC-1: $\beta = -0.44$, $t = -2.32$, $P = 0.038$), whereas PC-5 approached significance ($\beta = -0.39$, $t = -2.09$, $P = 0.058$). All other PCs were not significantly related with AI and were not included in the final model. Thus, according to correlations of chemical compounds with the PCs, male *I. monticola* with higher AI had femoral secretions with relatively higher proportions of hexadecanol, four steroids (cholesterol, cholestan-3-one, campesterol and sitosterol) and two fatty acids (dodecanoic and hexadecanoic acids) (PC-1) (Figure 2a), and with higher proportions of octadecanol (PC-5) (Figure 2b). A similar analyses considering time spent by female *I. monticola*' on paper strips scent marked by these males showed similar results ($R^2_{\text{model}} = 0.45$, $F_{2,14} = 5.78$, $P = 0.01$; PC-1: $\beta = -0.47$, $t = -2.41$, $P = 0.029$; PC-5: $\beta = -0.47$, $t = -2.39$, $P = 0.031$).

'Attractiveness indexes' of male *I. cyreni* were significantly and positively correlated with PC-5 resulting from the PCA for relative proportions of chemicals in their femoral secretions ($R^2_{\text{model}} = 0.43$, $F_{2,13} = 4.96$, $P = 0.024$; PC-5: $\beta = -0.49$, $t = -2.35$, $P = 0.034$). PC-4 approximated significantly ($\beta = 0.43$, $t = 2.09$, $P = 0.055$) and all other PCs were not significantly related with AI and were not included in the final model. Thus, according to correlations of chemical compounds with the PCs, male *I. cyreni* with higher AI had femoral secretions with more ergosterol (PC-5) (Figure 2c), more dehydrocholesterol and less squalene (PC-4) (Figure 2d). However, the analyses of time spent by female *I. cyreni* on paper strips scent marked by males did not show any significant correlation with any PC.

Males of both species that were more attractive to females had a better immune response (CMI) both when considering the 'attractive index' of males (*I. monticola*: $r = 0.62$, $F_{1,15} = 9.57$, $P = 0.007$; *I. cyreni*: $r = 0.55$, $F_{1,14} = 6.14$, $P = 0.026$) (Figure 3) or time spent by females on paper strips scent marked by these males (*I. monticola*:

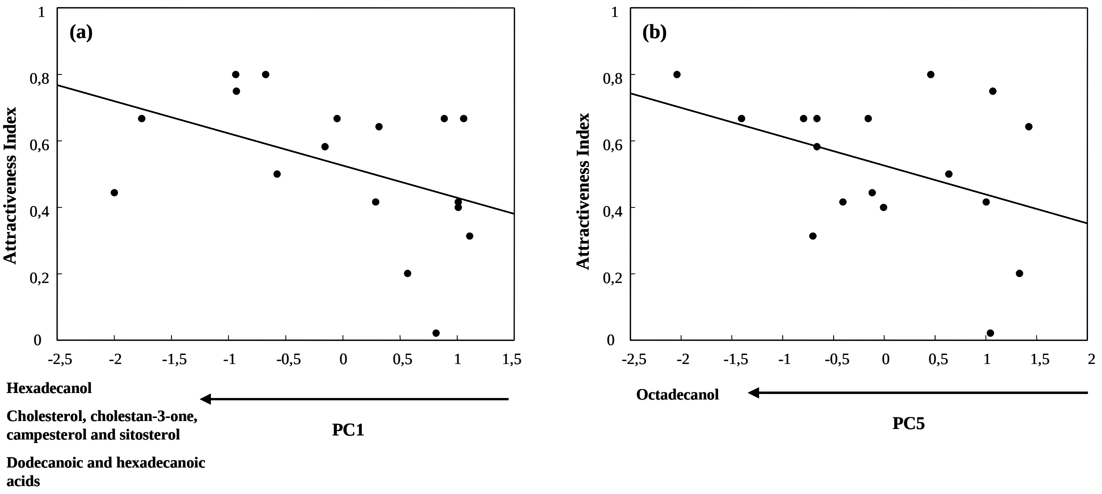
$r = 0.64$, $F_{1,15} = 10.73$, $P = 0.005$; *I. cyreni*: $r = 0.56$, $F_{1,14} = 6.57$, $P = 0.022$).

Discussion

Both male–male competition and female choice have been subject to considerable research, and understanding of both processes is well developed. By contrast, their interaction has received little attention and is poorly understood (but see Fitze et al. 2008). Recent studies suggest that caution should be exercised before assuming that traits selected in male–male competition are also preferred by females (reviewed in Qvarnström and Forsgren 1998; Wong and Candolin 2005). However, to our knowledge, our study is the first to examine whether females of two closed related species showed preferences for male signal traits with different information content.

Our results are in agreement with the hypothesis that male secondary sexual characters both function in male–male competition and as cues for female choice in more territorial species with lower population densities (*I. monticola*) but not in less territorial species with higher population densities (*I. cyreni*).

IBERIAN ROCK LIZARD ***(I. monticola)***



CARPETANE ROCK LIZARD ***(I. cyreni)***

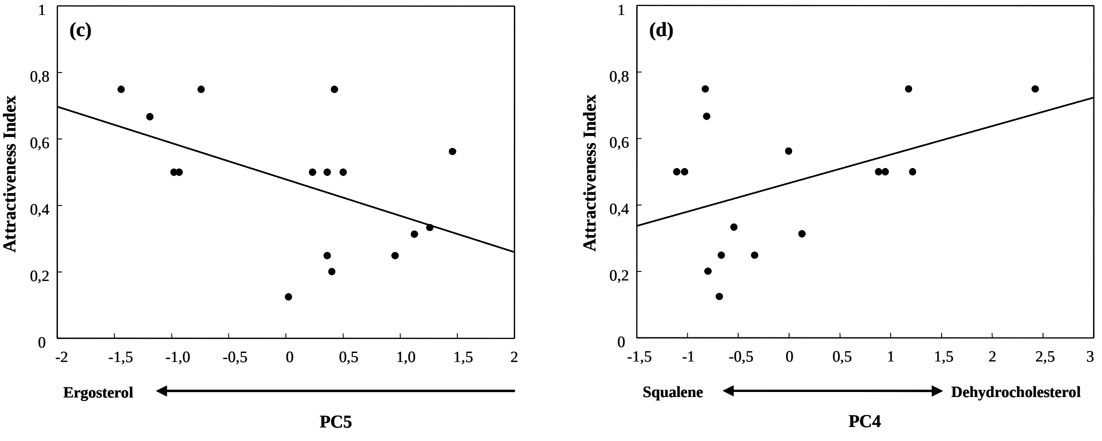


Fig. 2 Relationships between male 'attractiveness index' and PC- scores describing characteristics of chemical compounds in femoral gland secretions of (a) PC-1 or (b) PC-5 from males *I. monticola*, and (c) PC-5

Mating system differences

Head dimorphism is higher in *I. monticola* than in *I. cyreni*, which suggests that male-male competition is higher in *I. monticola*. Sexual dimorphism has been used widely as an indirect measure of sexual selection (e.g., Ord *et al*., 2001) because there is strong empirical evidence that sexual dimorphism often results from competition over mates and other resources (Andersson, 1994). Comparative studies have also demonstrated associations between sexual dimorphism and mating system (Figerola & Green, 2001), population density (Stamps *et al*., 1997) and territoriality (Cox *et al*., 2003). In lizards, sexual dimorphism in both body and head size has been shown to be primarily the result of intrasexual selection (Vitt & Cooper, 1985; Braña, 1996; Herrel *et al.,* 1999; Gvozdík & VanDamme, 2003).

Microhabitat availability and microhabitat preferences corresponded in *I. cyreni*, but not in *I. monticola*. Both Iberian rock lizard species selected microhabitats closed to rocks to thermoregulate and to seek refuge (Martín 2005a,b), however there were between-species differences in availability of preferred microhabitats. The habitat of *I. cyreni* had higher cover of refuges than in the habitat of *I. monticola*, which has more cover of grass and tall bushes (i.e. less appropriate areas to thermoregulate). Therefore, the available appropriate habitats for male and female *I. monticola* were scarcer than for *I. cyreni*, and hence may be more intensively defended by territorial *I. monticola* males. Different levels of resource availability in different areas are likely to affect the potential reproductive rates of both sexes (Ahnesjö et al. 2001) and hence the mechanisms of sexual selection (Lindström 2001). Some recent theoretical models suggest a role for the influence of habitat in the use and success of alternative male mating tactics (Hazel et al. 2004). If different species experience different environments, different male mating tactics may have evolved in different mating systems (Lukasik et al. 2006). Thus, differences in head dimorphism and habitat resources availability (i.e. refuges) suggest that *I. monticola* may be more territorial than *I. cyreni*, and signalling dominance may be advantageous for male *I. monticola* that need to defend the scarcer better territories available in their habitat. Moreover, male *I. cyreni* face very different selective pressures than *I. monticola*, because experience reduced opportunities for thermoregulation and activity, but can reach high population densities as a result of seasonal peaks in productivity and/or a scarcity of competitors or predators. Larger/older male *I. cyreni* are more active and dominant over smaller/younger ones, and there is extensive overlapping among male home ranges (Aragón et al. 2004). This mating system would allow the coexistence of alternative mating strategies, as suggested by the successful matings obtained by putative ‘‘transients’’ and by the fact that large males did not monopolize females (Salvador et al. 2008).

Female preferences

We found that female *I. monticola* preferred to associate with male chemical signals with relatively higher proportions of chemicals signalling dominance in this species (i.e. hexadecanol and octadecanol; Martín et al. 2007), whereas female *I. cyreni* did not show preference for male chemicals signals with higher proportions of chemicals related to dominance (i.e. cholesterol; Martín and López 2007), but did prefer male chemicals related with the immune system quality (i.e. dehydrocholesterol and ergosterol; Martín and López 2006a). In territorial species, successful males with large armaments may have a higher reproductive success as a result of competitive exclusion (e.g., *Lacerta agilis,* Olsson 1994). This may often be the case as dominant males probably pay lower costs of signalling than subdominant males and dominance often correlates with direct benefits, such as male genetic quality (Alatalo et al. 1991). Male-male competition could increase the honesty of signalling and, thus, facilitate female choice (Berglund et al. 1996). In these species, females that mate with dominant males may gain direct benefits from male defended resources (Candolin and Voigt 2001) or indirect benefits if competitive ability is heritable and her sons enjoy similar competitive ability (e.g. Moore 1990; Alatalo et al. 1991). Indeed, some studies have shown that the size of male secondary sexual characters, used in both male–male competition and female choice, can predict offspring survival (Jennions and Petrie 1997; Sheldon et al. 1997). However, several experimental studies have shown that females seem to base their mate choice mainly on the quality of the territory or nest site rather than on male traits (Alatalo et al. 1986; Hews 1990). Then although female attraction to essential resources could be the reason why males compete for access to such resources, male dominance *per se* does not need necessarily be used as an important cue in female choice. On the contrary, our study showed that females may be attracted to dominant males based exclusively on male chemical signals and not in territory resources in the territorial species (*I. monticola*).

It is largely unknown whether the balance between genetic benefits and costs of dominance is frequency dependent or varies with environmental conditions. Interspecific differences in mating system may promote different mating costs and benefits to females and dominance might not always be a reliable indicator of high genetically determined viability. In less territorial species, male dominance does not predict mate quality, and there may be fitness costs associated with choosing a dominant male (Qvarnström and Forsgren 1998, Holland and Rice 1999). For example, in lizards, dominance related traits such as aggression and fighting ability could have negative effects on female fitness and reproductive success, such as increased risk of injury when density is high (Fitze et al. 2008). In the less territorial species, density is high (220-328 ej/ha, Martín and Salvador 1997) so male harassment is expected to be high, too. Thus, if the interaction between males and females does not lead to a genetic covariance between male dominance and female preferences, females are expected to evolve resistance to male coercion, in an attempt to minimize the direct costs suffered (Kokko 2005) and prefer other male traits unrelated to dominance. On concordance, our results showed that female *I. cyreni* preferred to associate to male chemical signals with traits unrelated with dominance. Interestingly, in male *I. cyreni*, reproductive investment in morphological traits that confer dominance (i.e. head size) might lead to a low probability of survival by decreasing investment in other traits that affect locomotor performance (i.e. limb symmetry) (López and Martín 2002b). Moreover, more active males gained access to more females by overlapping home ranges of a larger number of females (Aragón et al. 2001) and reproductive success of male *I. cyreni* is explained by activity level but not by dominance (Salvador et al. 2008). The physical condition of males, which may enable them to be more active and search more intensively for receptive females (Hofmann and Henle 2006), may be a significant predictor of reproductive success in *I. cyreni* (Salvador et al. 2008). A better immune system could allow males to maintain a better physical condition and longer life span which, given the explosive nature of the breeding season in montane environments (only 2–3 weeks), may also increase male fitness. Therefore, selecting dominant males,

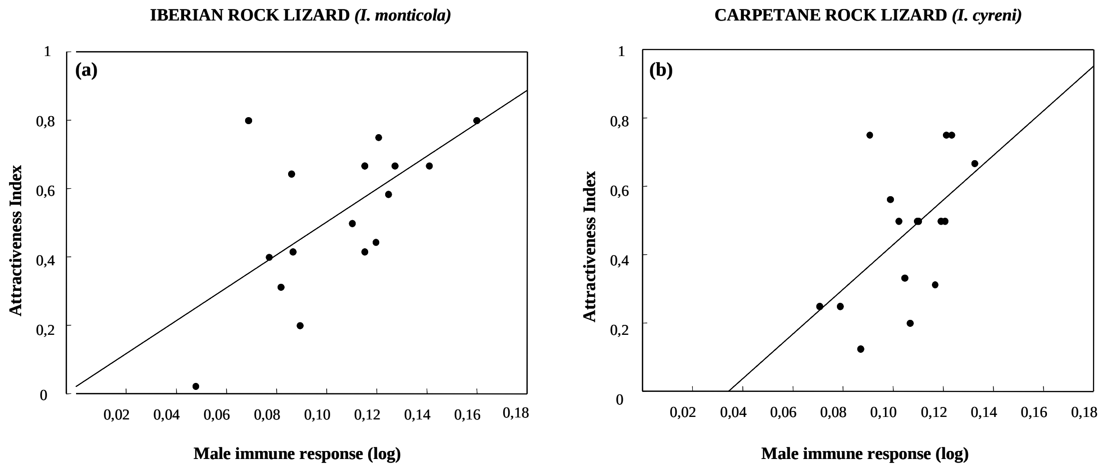


Fig. 3 Relationships between male 'attractiveness index' and T-cell mediated immune response (CMI) of males (a) *I. monticola* or (b) male *I. cyreni*.

female *I. cyreni* may not obtain higher fitness, but may be advantageous to prefer other male signal traits which honesty signalling male quality (for example, immune response). Thus, female *I. cyreni* may obtain indirect benefits selecting males with a better immune system and hence better physical condition and longer life span if male reproductive success is heritable.

Although females of both species of Iberian rock lizards seem to use different signal traits with different information content to select mates, both species prefer to associate to chemical signals of males with a better immune response, which may reflect male quality. This suggests that different mechanisms are involved to ensure honesty signalling in each species. For signals to reflect accurately the desired fitness gains to females, theory suggests that they must be costly to produce or maintain (honest) so that low-quality males cannot express as exaggerated signals as high-quality males (Zahavi 1975). In the species that uses the same trait to signal fighting ability and to attract mates (*I. monticola*), male-male competition could operate as an additional factor to ensure honest signalling of condition since faking quality in a competitive setting could potentially be quite costly if condition is subsequently put to the test in a fight (Berglund et al. 1996). Success in male contests depends on male condition and overall health in *I. monticola* (Martín et al. 2007), so dominant males may have better immune system. Thus, competition can actually facilitate female choice by ensuring that traits which simultaneously reflect dominance and fitness gains to females are signalled honestly in *I. monticola*. *I. monticola* female associated to chemical signals of dominant males, indirectly selected males with better immune system, reflecting male quality.

If fighting ability does not reflect the benefits that female *I. cyreni* seek, other mechanisms may be involved in ensure honesty of preferred male signal traits. Previous studies showed that the steroids implicated in female *I. cyreni* preferences for male chemical signals are important keys in many metabolism routes as precursors of signalling molecules with potent biological activity (Martín and López 2006b). Dehydrocholesterol is a precursor for vitamin D_3, and is often found in the skin, where it will transform into vitamin after exposition to sun ultraviolet (UV) B irradiation (Fraser 1995; Ferguson et al. 2005). Vitamin D_2 is similar but has to be acquired from the diet, as only fungus can convert it from the UV irradiation of ergosterol (Hay and Watson 1977). Allocating dehydrocholesterol (= provitamin D_3) to femoral secretions male lizards will need to divert vitamin D_3 from metabolism (Martín and López 2006b), whereas ergosterol in secretions might be directly related to the amount of vitamin D_2 that a lizard has been able to acquire from the diet. Thus, variations of these chemicals in male chemical signals might be costly, especially for low-quality individuals (e.g. those with a worse health state or less symmetric) that would not be able to obtain from the diet enough vitamin D (Martín and López 2006b). Moreover, other studies show that individual *I. cyreni* lizards with a worse health state are shyer and hide for longer in refuges after a predator approach (López et al. 2005), and that ‘inferior’ subordinate males were active for less time during the day (Martín and López 2000b). Therefore, these presumably low-quality individuals would have less time available for foraging and acquiring vitamin D from the diet and also for looking for females, reducing their reproductive success (Salvador et al. 2008). Then, by preferring male chemical signals with higher proportions of dehydrocholesterol and ergosterol, female *I. cyreni* may increase the probability of mating with males with better immune system (i.e. better quality males).

Conclusions

In summary, differences in mating systems may promote mate preferences for different male traits. Moreover, differences in the mating system may promote that different mechanisms are implicated in honesty of male signals (Endler 1993). Little is known about correlational female mating preferences and signal information content, but testing for such preferences is essential for understanding both the nature of female preferences and how they affect the evolution of male signals. Signal design depends on environment characteristics (Endler 1992), but environment includes both social and physical environment. Hence, social environment, such as mating system, may also drive signal design and between-species divergence. This hypothesis would typically be approached with a broad phylogenetic comparison investigating how females from different populations or closed related species weigh different traits and whether their relative importance varies with mating system. Such studies will provide new insights not only into the evolution of the information content of male signals but also into multiple signalling, male mating tactics and female preferences in general.

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Supplement 1. Principal components analysis for microhabitat available and used by adult lizards *I. monticola* and *I. cyreni*. Bold values are significant at *P*<0.0001.

	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6
Cover						
Small rocks	0.07	**-0.72**	-0.13	0.25	-0.02	0.00
Medium rocks	0.12	**-0.79**	0.13	0.16	0.07	-0.18
Large rocks	0.07	0.16	-0.02	**-0.90**	-0.20	0.02
Rocky outcrops	0.11	**0.78**	-0.05	0.31	0.04	-0.10
Bare sandy soil	-0.04	0.01	-0.01	-0.13	0.06	**0.77**
Grass	**-0.33**	0.46	0.12	**0.37**	-0.24	**0.43**
Leaf litter	**-0.39**	0.08	-0.03	0.07	**0.69**	-0.06
Canopy tree	0.05	-0.17	0.14	0.12	**0.81**	0.23
Sunny locations	**0.33**	-0.03	0.04	0.00	**-0.65**	0.24
Grass contacts						
5 cm	-0.18	0.09	**0.35**	**0.42**	-0.13	**0.65**
10 cm	0.08	0.12	**0.66**	0.09	0.13	**0.52**
25 cm	-0.01	-0.20	**0.84**	-0.03	0.19	0.20
50 cm	0.03	0.06	**0.81**	0.01	-0.16	-0.21
Bush contatcts						
5 cm	**-0.92**	-0.06	0.02	0.01	0.14	0.06
10 cm	**-0.95**	-0.04	0.00	0.09	0.09	0.06
25 cm	**-0.95**	0.02	-0.09	0.10	0.08	0.00
50 cm	**-0.68**	**0.39**	0.01	-0.13	0.31	-0.05
75 cm	**-0.48**	**0.46**	0.11	**-0.35**	**0.33**	0.09
100 cm	**-0.42**	**0.44**	0.11	**-0.35**	**0.34**	0.12
Distance to refuge	0.16	**0.58**	-0.12	-0.05	-0.17	**0.54**
Eigenvalue	4.4825	3.0726	2.5521	1.6015	1.3551	1.1574
% Total variance	23.592	16.171	13.432	8.429	7.132	6.091

Capítulo 5

DIFERENCIAS MICROGEOGRÁFICAS EN LAS SEÑALES QUÍMICAS DE LOS MACHOS Y EN LAS PREFERENCIAS DE LAS HEMBRAS POR DIFERENTES COMPUESTOS RELACIONADOS CON EL SISTEMA INMUNE: CONSECUENCIAS PARA LOS PROCESOS DE ESPECIACIÓN

La hipótesis de la especiación por selección sexual argumenta que la divergencia en las preferencias de las hembras por las señales de los machos locales puede causar un fuerte aislamiento precópula. Dado que las presiones de la selección pueden variar geográficamente, pueden aparecer patrones complejos cuando la asociación entre preferencias y señales sexuales se considera tanto entre poblaciones, como dentro de las mismas. El objetivo de este estudio ha sido explorar en dos poblaciones aisladas de lagartija carpetana (*Iberolacerta cyreni*) las relaciones entre las características de las señales químicas de los machos, su calidad individual (medida como respuesta inmune) y las preferencias de las hembras. Nuestra hipótesis de partida es que el aislamiento entre ambas poblaciones podría promover divergencias en las señales químicas de los machos y en las preferencias de las hembras, lo que podría llevar a un aislamiento reproductivo. Nuestros resultados mostraron variación entre poblaciones en las señales químicas de los machos y en las preferencias de las hembras. Las hembras prefirieron asociarse con las señales químicas de los machos con mejor respuesta inmune, lo que sin embargo, estaba correlacionado con diferentes rasgos de las señales de los machos en cada población. Así, al menos en una de las poblaciones, un proceso indicador de selección sexual divergente podría haber llevado a una coevolución de los rasgos de las señales de los machos y las preferencias de las hembras. Es más, las hembras prefirieron asociarse con los olores de los machos simpátricos, lo que parece estar determinado por las diferentes características de las señales químicas de los machos y podría promover el aislamiento sexual y la especiación ■

MICROGEOGRAPHIC DIFFERENCES IN MALE CHEMICAL SIGNALS AND FEMALE PREFERENCES FOR DIFFERENT COMPOUNDS RELATED TO MALE IMMUNE RESPONSE: CONSEQUENCES FOR SEXUAL ISOLATION

The hypothesis of speciation by sexual selection argues that divergent female preferences for signals of local males may cause strong premating isolation. Given that selection pressures may vary geographically, complex patterns may emerge when the association between preferences and mating signals is considered both within and among populations. The aim of this study is to explore in two isolated populations of *I. cyreni* lizards the relationship between male chemical signal characteristics, individual quality (measured as the immune response) and female preferences. We hypothesized that isolation between both populations could promote divergence in males' chemical signals and female preferences, which might lead to reproductive isolation. We found variation in male chemical signals and in female preferences between populations. Females preferred to associate with chemical signals of males with better immune response, which was, however, correlated with different traits of male signals in each population. Thus, at least in one of the populations, an indicator process of divergent sexual selection might lead to covariation of male signal traits and female preferences. Moreover, females preferred to associate with scent of sympatric males, which seems determined by different characteristics of male chemical signals and might promote sexual isolation and speciation.

Keywords: signal evolution, population divergence, indicator processes, speciation by sexual selection, lizards.

Understanding the variation between populations in male sexual signals and female preferences is crucial to theories concerning the origin of sexual isolation by sexual selection or other processes. Closed related species differ most in traits involved with mating success rather than any species, so the evolutionary diversification of many animal taxa may be facilitated by geographical variation in mating signals (West-Eberhard 1983; Endler 1992; Wells and Henry 1998; Foster 1999; Coyne and Orr 2004). Sexual selection has a reputation as a major cause of speciation, one of the most potent forces driving reproductive isolation (Ritchie 2007). However, a critical component of models of speciation based on sexual selection is that reproductive isolation results when both male mating signals and female preferences coevolve within populations; evolutionary changes that involve only one component will not lead to isolation and speciation (Ritchie 2000; Boughman 2001). Some studies emphasize the role of genetic drift (Coyne and Orr 2004), stochastic variation (Schluter 2000) or reinforcement (Coyne and Orr 1997). However, most of these studies deal with already well differentiated species. Comparison of preferences with signal traits for closely related species or populations can reveal the extent to which signals have responded to selection exerted by preferences. If there is sufficient variation, assortative mating is expected to generate a positive covariance between important male mating traits and female preferences within populations, and coevolution between these traits can lead to the divergence of mate recognition systems between populations and, thereby, cause sexual isolation and speciation (Lande 1981, 1982). However, covariation between signals and preferences among populations is surprisingly scarcely reported.

Male signals involved in sexual selection often vary geographically. Divergence of mate signals and preferences likely occurs because geographically distinct populations experience different environmental conditions that can influence the strength or direction of sexual selection (Endler 1992). Thus, complex patterns may emerge when the association between preferences and mating signals is considered both within and among populations.

Iberian rock lizards comprise several closely related species and subspecies that occupy different isolated high mountain areas in the Iberian Peninsula (Mayer and Arribas 2003; Carranza et al. 2004). In the Guadarrama Mountains (Central Spain), *Iberolacerta cyreni* (formerly *Lacerta monticola cyreni*) is restricted to open rocky habitats above 1700 m altitude. Mountain topography and historical climatic changes have led to different isolated populations, which are geographically close but separated by the presence at lower altitudes of surrounding pine forests where this lizard does not occur. This fragmented population system provides isolated lizard populations that differ in altitude and orientation, which may imply different environmental conditions. In reptiles, the chemical senses play important roles in intraspecific communication, and chemosensory recognition may be especially important in speciation processes (Cooper and Vitt 1987; Shine et al. 2002; LeMaster and Mason 2003). Chemical signaling is also well-developed in this species, where scent marks from femoral gland secretions of males indicate individual quality and convey information about social status (Aragón et al. 2001; López et al. 2006; Martín and López 2007) or about traits used by females in mate choice (Martín and López 2000, 2006a,c; López et al. 2002, 2003). Chemical signals are important for species recognition in many taxa and, like other signals favored by sexual selection, they may play important roles in speciation (Phelan and Baker 1987; McLennan and Ryan 1999; Wyatt 2003). Although the topic of chemical signals differentiation and its potential role in reproductive isolation and allopatric speciation is one of high interest in evolutionary ecology, very few studies have addressed this issue in lizards (Martin and López 2006b).

We hypothesized that isolation and environment differences between two isolated populations of *I. cyreni* lizards may promote signal divergence. The aim of this study is to explore whether signal divergence may affect to female preferences and signal indication of individual quality (measured as the immune response) and the implications on sexual isolation and eventual speciation processes. Firstly we analyzed chemical signal composition and looked for correlations between female preferences and male signal traits with laboratory association trials. If female preferences coevolve with male signals, it may lead to an eventual sexual isolation process. However, the evolutionary impact of such geographical variation of male mating signals may depend critically on patterns of male signal perception and population discrimination by females. If females *I. cyreni* discriminate among geographical signal variations, then gene flow can be reduced (Wells and Henry 1998; Irwin 2000). Secondly we conducted female association trials to allopatric vs. sympatric male chemical signals to examine whether differences in males' chemical signal may be discriminated by females from both populations.

Sexual selection may lead to variation in signal traits without leading to speciation, for example where signal transmission evolves to maximize detectability in different environments through a 'sensory drive' process (Endler and Basolo 1998). However, where sexual selection by mate choice has exerted a dominant influence on the evolution of mating signals, we should expect an interpopulation correlation between preferences and signal traits under standardized environmental conditions in the laboratory. Aside from sensory drive processes, geographical variation of female preferences is expected from other kinds of mate-choice models such us Fisher's runaway process, indirect-benefit selection or indication models ('good genes', handicap models) and direct-benefit selection (parental care, nuptial gifts) (Endler and Basolo 1998). There are not direct-benefits in lizards, but several studies show the existence of indirect-benefit selection in *I. cyreni* lizards (Martín and López 2000, 2006a,c; López et al. 2002, 2003). Previous studies show that female *I. cyreni* preferred to associate with chemical signals of males with better immune response (CMI) (Martín and López 2006a). In spite of the signal population divergence, male signals should still be functionally significant in mate choice and female preferences should coevolve with them. Then, thirdly we explored whether female *I. cyreni* prefer to mate with males with better CMI in both populations an whether individual CMI is still signaling in both populations by the same, or different chemical compounds.

Methods

Study animals

During April 2004, we captured by noosing 12 adult male and 18 female *I. cyreni* lizards at the lower part of 'Montón de Trigo' (40°48'N, 4°4'W; 1950 m altitude), and 11 adult male and 18 female at "Cueva" (40°47'N, 4°1'W; 1750 m altitude); two localities in the Guadarrama Mountains. These two populations are 17 km apart, and are isolated by a lower altitudinal area (below1700 m) with different habitat conditions (dense pine forest) where no stable population of Iberian rock lizards is found. Within each population, we captured lizards in different places over large areas (5 km^2) to ensure that individuals had not been in previous contact, which might affect their responses (López and Martín 2002).

Lizards were individually housed at "El Ventorrillo" Field Station, 5 km from the capture sites of Guadarrama populations, in outdoor 60 x 40 cm PVC terraria containing rocks for cover and absorbent paper as substrate, which was used to collect femoral gland secretions of males (see below). Water and mealworm larvae dusted with multivitamin powder were provided ad libitum. The photoperiod and ambient temperature were those of the surrounding region. Lizards were captured during two consecutives days and were held in laboratory conditions one week before testing to allow acclimation. Lizards from both populations spent in captivity the same number of days before testing. To avoid that lizards had contact with the scent stimuli before they were tested, terraria with lizards of different populations and different sexes were housed separately.

We used the phytohaemagglutinin (PHA) injection test (a delayed-type hypersensivity test) to assess the T cell-mediated immune (CMI) response in vivo (Smits et al. 1999; Belliure et al. 2004). We measured with a pressure-sensitive spessimeter the thickness (to the nearest 0.01 mm) of the right hindlimb foot pad at a marked point before and after 24 h of injecting 0.02 mg of PHA dissolved in 0.01 ml phosphatebuffered saline at this point. We calculated the CMI response as the difference between pre- and postinjection thickness measures (Smits et al. 1999). The only appreciable effect of the PHA injection was a slight swelling of the skin, which disappeared after 48 h. No lizard showed any sign of stress or pain in these tests or during the trials. Lizards were returned to their exact capture sites at the end of experiments.

Table 1 Lipophilic compounds found in femoral gland secretions of male lizards *I. cyreni* of two close but isolated populations ('Cueva' and 'Trigo'). The relative amount of each component was determined as the percent of the total ion current (TIC) and reported as the average (± 1S).

Compound	Cueva	Trigo
Steroids:		
Cholesta-2,4-diene	0.11 ± 0.04	0.08 ± 0.07
Cholesta-4,6-dien-3-ol	0.16 ± 0.05	0.19 ± 0.08
Cholesta-3,5-diene	0.23 ± 0.07	0.30 ± 0.09
Cholesterol	71.36 ± 4.59	69.30 ± 4.66
Cholesta-5,7-dien-3-ol	0.06 ± 0.09	0.09 ± 0.09
Ergosta-5,22-dien-3-ol	0.18 ± 0.07	0.22 ± 0.10
Ergosterol	0.69 ± 0.25	0.85 ± 0.45
Stigmasta-5,24(28)-dien-3-ol	0.93 ± 0.45	0.84 ± 0.20
Campesterol	6.69 ± 2.22	5.62 ± 1.09
4-Methyl-cholest-7-en-3-ol	0.01 ± 0.01	0.01 ± 0.01
Ergosta-5,8-dien-3-ol	1.40 ± 1.04	1.46 ± 0.57
Lanost-8-en-3-ol	0.79 ± 0.33	0.73 ± 0.34
2,2-Dimethyl-cholest-8(14)-en-3-ol	0.05 ± 0.07	0.04 ± 0.08
γ-Sitosterol	2.32 ± 0.81	2.01 ± 0.61
24-Propylidene-cholest-5-en-3-ol	0.23 ± 0.09	0.23 ± 0.07
4,4-Dimethyl-cholest-7-en-3-ol	0.19 ± 0.14	0.20 ± 0.09
4,4-Dimethyl-cholesta-5,7-dien-3-ol	2.39 ± 1.04	2.49 ± 0.79
Carboxylic acids and their esters*:*		
Nonanoic acid	0.01 ± 0.01	0.01 ± 0.01
Decanoic acid	0.01 ± 0.01	0.01 ± 0.01
Dodecanoic acid	1.54 ± 0.55	2.03 ± 0.93
Tetradecanoic acid	0.09 ± 0.03	0.12 ± 0.04
Pentadecanoic acid	0.04 ± 0.02	0.05 ± 0.03
Hexadecanoic acid	4.87 ± 1.53	5.57 ± 2.09
Hexadecenoic acid	0.25 ± 0.09	0.25 ± 0.12
Hexadecanoic acid, ethyl ester	0.03 ± 0.04	0.11 ± 0.24
Heptadecanoic acid	0.04 ± 0.03	0.04 ± 0.04
Octadecanoic acid	1.12 ± 0.37	1.21 ± 0.55
Octadecenoic acid	3.01 ± 1.07	4.13 ± 1.67
Octadecadienoic acid	0.52 ± 0.31	0.73 ± 0.44
Octadecenoic acid, methyl ester	0.18 ± 0.11	0.25 ± 0.18
Eicosanoic acid	0.24 ± 0.13	0.23 ± 0.13
Eicosatetraenoic acid, methyl ester	0.03 ± 0.05	0.04 ± 0.07
Docosanoic acid	0.01 ± 0.01	0.01 ± 0.01
Alcohols:		
Octadecanol	0.02 ± 0.03	0.04 ± 0.05
Eicosanol	0.02 ± 0.04	0.25 ± 0.18
Docosanol	0.04 ± 0.05	0.09 ± 0.07
Tetracosanol	0.01 ± 0.01	0.01 ± 0.01
Hexacosanol	0.01 ± 0.01	0.01 ± 0.01
Others:		
Squalene	0.10 ± 0.04	0.14 ± 0.06
4-Hydroxy-hexadecanoic acid γ-lactone	0.08 ± 0.04	0.08 ± 0.05

Analyses of femoral gland secretions

Immediately before female association trials, we extracted femoral gland secretion of males by pressing with forceps around the femoral pores. We analyzed lipophilic compounds in femoral secretions by using a ThermoQuest Trace 2000 gas chromatograph-mass spectrometer (GC-MS) fitted with a 30 m column (Supelco, Equity-5) (for details of analytical procedures see López et al. 2006). Initial identification of compounds was done by comparison of mass spectra in the NIST 1998 mass spectral library, and later confirmed with authentic standards. The relative amount of each component was determined as the percent of the total ion current (TIC). Areas of the peaks that represented >0.05% relative peak area (which together represent 96.8% of TIC area) were transformed following the formula of Aitchison (1986) and used as variables in a principal component analysis (PCA) (for similar analyses see Dietemann et al. 2003; López et al. 2006). The six principal components (PC) extracted (which explained 65.2% of variance; Supplement 1) were used as independent variables in one way ANOVAs to determine whether the two Guadarrama populations of *I. cyreni* differed in the relative abundances of compounds.

Female association trials

During April, to obtain the scents from male lizards, we had placed in each male's cage several absorbent paper strips (35x10 cm) fixed to the floor, and left them there for ten days. Experiments were carried out during the mating season (May-June). Papers were removed to be placed in females' cages immediately before each experiment. Females' cages (60x40 cm) had a central refuge, two basking tiles at each side, and a water recipient. At the beginning of each experiment (0700 h), when females were still inactive and hidden in the refuge, we fixed on each basking tile one paper strip from a male from the same population than the female, and another paper from a male from the other population on the other basking tile. The individual males tested were randomly determined and the scent papers from "same" versus "other" males were switched between the two tiles for each of the seven trials per female. Each female was tested on seven different days, with papers from fourteen different males (seven different pairs consisting of a male from their own population and a male from the other population). Each paper strip was used only in one test to avoid contamination.

Females were monitored each 10 min (between 1000 and 1400 h) from a hidden point, and their locations in the cages were recorded. These locations were considered to be representative of females' space use of the cages. If a female was located on either of the two papers, she was considered as having chosen temporally that particular paper, whereas if she was located in a non specific position (e.g., central refuges), we assumed that she had made no choice (for a similar procedure see Martín and López 2000, 2006a; Olsson et al. 2003; López et al. 2003). At the end of each trial, the papers were removed and the cage was thoroughly rinsed with clean water to eliminate odors of precedent trials.

We compared the relative time (relative number of observations) that each female spent on each paper with a two-way repeated measures ANOVA with type of male (male from her own population *vs.* male from the other population) and day of the trial, both as within factors, and the population of the responding female ('Cueva' *vs*. 'Trigo') as a between factor. We included the interactions in the model to analyze whether responses to the different scents differed as a function of the population of origin of the responding female.

Pairwise comparisons were planned using Tukey's honestly significant difference tests (Sokal and Rohlf 1995).

To examine population differences in female preferences for characteristics of males chemical signals, we used general regression models (GRM) (using Statistica 6.0 software) with the relative time that a female spent on the paper scent marked by the male from her own population (time on paper of own population male / time on papers of both males) and the six principal components (PC) resulting from the PCA for relative proportions of chemicals in femoral secretions of males (see above) or CMI as potential predictors of the female preference. Data normality was verified by Shapiro-Wilk's tests, and tests of homogeneity of variances (Leveane's test) showed that variances were not significantly heterogeneous after transformation. We developed the tests within each population separately because interpopulation differences in relative proportions of chemicals did not comply with the assumption of homogeneous slopes between groups (Garcia-Berthou 2001; Engqvist 2005). To decide what variables best predicted female preference, we used a "best-subsets" approach in conjunction with stepwise methods (Neter et al. 1985). We chose the most parsimonious model as having the lowest Akaïke information criterion (AIC), and we checked its coincidence with the equivalent stepwise solution. Residuals from the final models were normally distributed (Grafen and Hails 2002).

Results

Chemical composition of males' femoral gland secretions

The lipophilic fraction of femoral gland secretions of male *I. cyreni* is a mixture of steroids (84.5% of TIC), and carboxylic acids ranged between C_9 and C_{22} and some of their esters (11.2%), but we found also five alcohols between C_{18} and C_{26} (0.25%), squalene (0.12%), and a furanone (0.08%) (Table 1). On average, the five most abundant chemicals were cholesterol (70.3%), campesterol (6.2%), hexadecanoic acid (5.2%), octadecenoic acid (3.6%), and 4,4-dimethyl-cholesta-5,7-dien-3-ol (2.4%).

All compounds were shared by all males from the two populations. However, there were significant differences in relative proportions of some shared compounds between populations (Table 1). These were due to significant differences in PC-1 (ANOVA, $F_{1,30} = 7.66$, $P < 0.01$), but not in other PCs ($P > 0.20$ in all cases). Thus, according to the correlations of compounds with the PC scores (Supplement 1), males from 'Trigo' had secretions with relatively higher proportions of docosanol and cholesta-5,7-dien-3-ol, and lower proportions of steroids such as campesterol and sitosterol, than males from 'Cueva'.

Female association trials

Time spent by females from 'Trigo' on paper strips scent marked by males from their own population was significantly and negatively correlated with PC-1 and PC-2 resulting from the PCA for chemicals in femoral secretions of males ($R^2_{\text{model}} = 0.85$, $F_{2,9} = 27.41$, $P = 0.0001$; PC-1: $\beta = -0.62$, $t = -5.01$, $P = 0.0007$; PC-2: $\beta = -0.60$, $t = -4.74$, $P = 0.001$; Fig. 1a). All other PCs were not significantly related with time and were not included in the final model. Thus, females of 'Trigo' spent more time on papers with scent of males that had femoral secretions with relatively higher proportions of docosanol and cholesta-5,7-dien-3-ol, and lower proportions of steroids such as campesterol and sitosterol (PC-1), and that had higher proportions of fatty acids such as hexadeacanoic, octadecanoic and octadecenoic acids (PC-2).

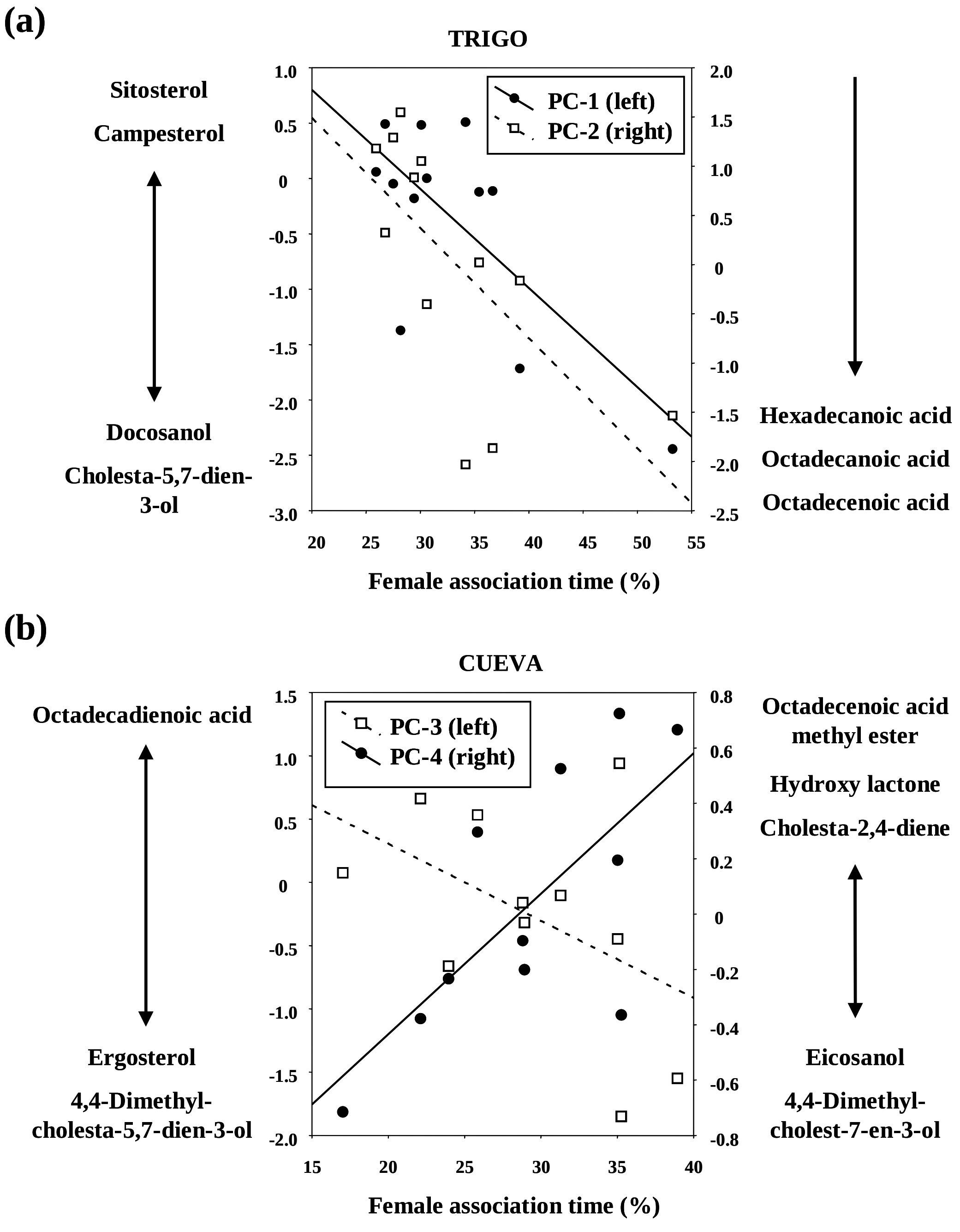

Fig. 1 Relationships between average percent time that female lizards *I. cyreni* spent on the papers with scents of individual males from their own population and PC-scores describing characteristics of chemical compounds in femoral gland secretions of these males from ‘(a) Trigo’ (filled circles: PC-1; open squares: PC-2) or (b) from ‘Cueva’ (open squares: PC-3; filled circles: PC-4).

Similarly, time spent females from ‘Cueva’ on paper strips scent marked by males from their own population was significantly and negatively correlated with PC-3 and positively with PC-4 for chemicals in femoral secretions (R^2_{model} = 0.74, $F_{2,8}$ = 15.44, P = 0.0017; PC-3: β = -0.53, t = -3.29, P = 0.01; PC-4: β = 0.76, t = 4.76, P = 0.001; Fig. 1b). Thus, females of ‘Cueva’ spent more time on papers with scent of males that had femoral secretions with relatively higher proportions of ergosterol and 4,4-dimethyl-cholesta-5,7-dien-3-ol and lower proportions of octadecadienoic acid (PC-3), and with higher proportions of octadecenoic acid methyl ester, the hydroxy lactone and cholesta-2,4-diene and lower proportions of 4,4-dimethyl-cholest-7-en-3-ol and eicosanol (PC-4).

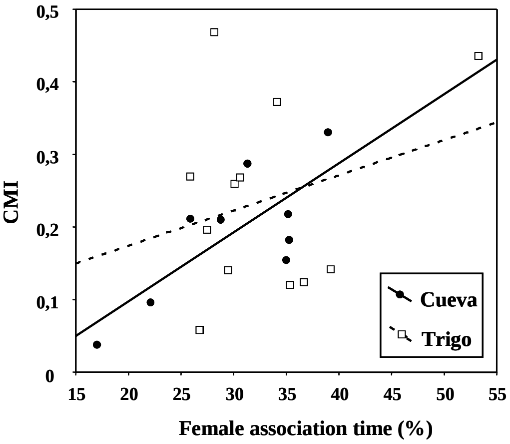

Fig. 2 Relationships between average percent time that female lizards *I. cyreni* spent on the papers with scents of individual males from their own population and T-cell mediated immune response (CMI) of these males from 'Trigo' (filled circles) or from 'Cueva' (open squares).

Females from both populations spent more time on papers with scent of males with higher immune response ('Trigo': $R^2_{\mathrm{model}} = 0.36$, $F_{1,10} = 5.77$, $P = 0.03$; 'Cueva': $R^2_{\mathrm{model}} = 0.58$, $F_{1,7} = 10.05$, $P = 0.01$) (Figure 2). However, males from 'Trigo' with higher CMI responses had femoral secretions with relatively higher proportions of docosanol and cholesta-5,7-dien-3-ol, and lower proportions of steroids such as campesterol and sitosterol (PC-1) ($r = -0.89$, $F_{1,10} = 41.05$, $P < 0.0001$; Fig. 3a), whereas all other PCs were not significantly related to CMI ($P > 0.40$ in all cases). In contrast, males from 'Cueva' with higher CMI responses had higher proportions of octadecenoic acid methyl ester, the hydroxy lactone and cholesta-2,4-diene and lower proportions of 4,4-dimethyl-cholest-7-en-3-ol and eicosanol (PC-4) ($r = 0.84$, $F_{1,7} = 17.06$, $P = 0.004$; Fig. 3b), whereas all other PCs were not significantly related to CMI ($P > 0.20$ in all cases).

There were significant differences between treatments (own or other population) in the number of observations of females from both populations on a determined stimulus paper strip (repeated measures two-way ANOVA: $F_{1,34} = 9.95$, $P = 0.0033$), and between days ($F_{6,204} = 3.01$, $P = 0.008$), but there were not significant differences in the overall responses of the two populations of females ($F_{1,34} = 0.40$, $P = 0.53$) (Fig. 4). Thus, females from both populations spent more time on paper strips scent marked by males of their own population. The interaction between treatment and population of the responding female approached significance ($F_{1,34} = 3.41$, $P = 0.07$). Thus, differences in preference for scent of males of their own population against males of the other population were greater, albeit not significantly, in females from 'Trigo' than in females from 'Cueva'. All the other interactions were not significant ($P > 0.59$ in all cases).

Discussion

Most mate choice processes can result in the coupling of the display trait and preference; that is, females exhibit preferences for traits that are possessed by males in their population (Houde and Endler 1990; Endler and Houde 1995). Positive assortative mating preferences and variation in male sexual morphology between populations has been reported in fishes (Tilley et al. 1990; Endler and Houde 1995; Knight and Turner 2004; Zuarth and Macías-Garcia 2006), insects (Butlin 1996; Ritchie 1996; McMillan et al. 1997), lizards (Kwiatkowski and Sullivan 2002), snakes (LeMaster and Mason 2003) and mammals (Pillay 2000). However, other studies found a lack of coordinated geographic variation between male signals and female preference in insects (Gray and Cade 2000; Simmons et al. 2001) or fishes (Hamilton and Poulin 1999). Our study indicates that there is substantial variation in the characteristics of male chemical signals and also in female signal traits preferences, within and between two geographically distinct *I. cyreni* populations. We found coordination between female preferences and male signals in ‘Trigo’, but not in ‘Cueva’ population, which may suggest a covariation between female preferences and male signals only in one population.

Previous studies show that female *I. cyreni* may select mates based on the chemical characteristics of femoral secretions of males (Martín and López 2000, 2006a,c; López et al. 2002, 2003). Modern treatments recognize that the processes of mate choice and species recognition are part of a continuum: females may discriminate against some males because they are of low quality or less stimulating, but when that process leads to discrimination between geographic races then it contributes to species recognition (Ryan and Rand 1993; Endler and Houde 1995; Endler and Basolo 1998; Littlejohn 1999). Female Iberian rock lizards avoid territories of non-preferred males (López et al*.* 2003). Males have relative small home range, although lizards usually move around to look for females (Aragón et al*.* 2004) and when males find a female often occur forced copulations, like occurs in other lizard species. Therefore, by remaining in a male territory (i.e. association to male chemical signals) a female may increase the probability of mating with that male. We found that females from ‘Trigo’ preferred to associate to scent marks of males with chemical characteristics that were precisely those that differed between populations, and, therefore, may explain that females from ‘Trigo’ preferred to associate to scent marks of males from their own population. Although most models of speciation based on sexual selection theory predict correlation between signals and preferences, some models predict that preferences and display traits will not always be correlated among populations (Endler and Basolo 1998; Wiens 2001); females may possess preferences for traits not possessed by males in their population (or viceversa, when the trait is present, but the preference is not) resulting in a preference-trait asymmetry among populations (e.g., Hill 1994; Arnold et al. 1996; Simmons et al. 2001). Our results showed that, at least in one of the populations, it might have undergone divergent sexual selection leading to coevolution of male signal traits

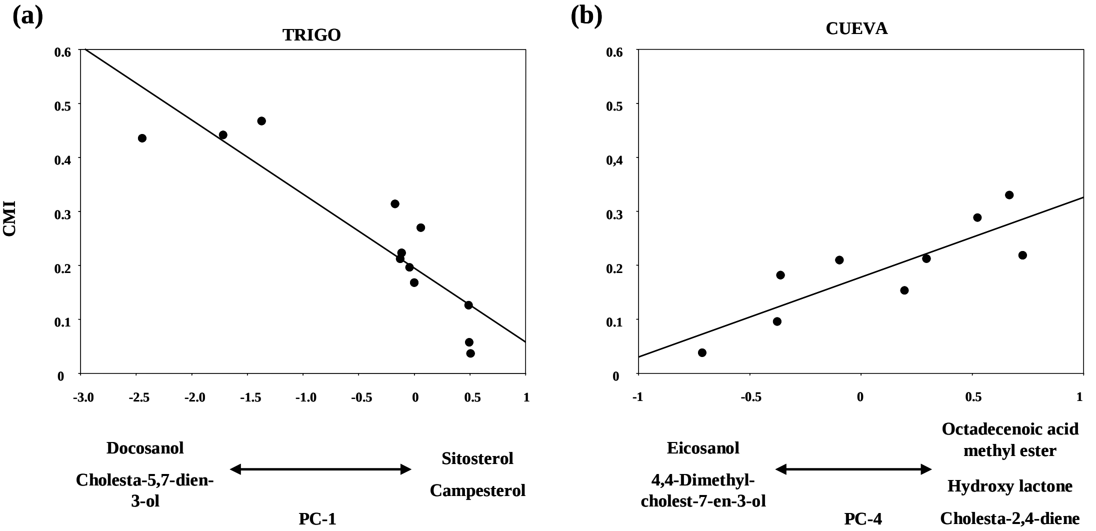

Fig. 3 Relationships between T-cell mediated immune response (CMI) and PC-scores describing chemical signal characteristics of male lizards from (a) ‘Trigo’ or (b) from ‘Cueva’.

and female preferences as predicted by most models of speciation based on sexual selection theory (Endler and Basolo 1998). The evolutionary impact of this geographical phenotypic variation of male *I. cyreni* mating signals also depends critically on patterns of signal perception and population discrimination by females. Between-population female association patterns to male chemicals showed that females *I. cyreni* spent more time on paper strips scent marked by males of their own population, which indicate population discrimination.

The concomitant assumption that change in species recognition systems arose owing to direct selection to avoid deleterious hybridization predominated early discussions about species recognition (i.e., reinforcement; Coyne and Orr 1997; Servedio and Noor 2003). However, when populations are isolated, reinforcement makes no sense, and other mechanisms should explain assortative preference. Much of the research in this area has taken place in the context of Fisher's runaway process, but other forms of sexual selection such as "good genes" or indicator processes would also generate this covariance between male signals and female preferences (Endler and Basolo 1998). For instance, immune response dependence of male traits can lead to accelerated and extended evolution of male signals (Lorch et al. 2003). It seems likely that this would also accelerate allopatric speciation, but this has not been modeled explicitly. In accordance with previous studies (Martín and López 2006a), we found that females from both populations preferred to associate with chemical signals of males with better immune response (CMI). However, CMI is correlated with different traits (i.e., different proportions of chemical compounds) of male signals preferred by females in each geographically distinct population. Therefore, although females from two different populations showed preference association patterns for different chemical characteristics of male signals, the relationship between these chemicals and the male 'quality' is maintained in both populations. Handicap processes ensuring signal honesty, or different evolutive pressures, may be diverged between both populations. The steroids are important keys in many metabolism routes as precursors of signalling molecules with potent biological activity. For instance, by allocating cholesta-5,7-dien-3-ol ('Trigo' males) or cholesta-2,4-diene ('Cueva' males) to femoral secretions male lizards will need to divert them from metabolism, whereas docosanol ('Trigo') or octadecenoic acid methyl ester ('Cueva') in secretions might be directly related to the amount of them that a lizard has been able to acquire from the diet. Thus, signals based on variations in these chemicals might be reliable and costly to produce, especially for low-quality individuals (those with a worse health state) that would not be able to obtain them from the diet (Martín and López 2006a). This suggests the potential role of these secretions as condition dependent honest advertisements (Grafen 1990; Kotiaho 2001). The question that arises is why different compounds of male chemical signals should be associated with CMI in each population.

It is still debated whether ornaments evolve because they are costly (Zahavi, 1975), or they become costly because their magnitude is exaggerated as a result of mate choice (Fisher, 1958). Both social and physical environment may drive the evolution and divergence signalling systems. Social environment include intraspecific interactions such as different female mate preferences and selective processes due to different evolutive pressures. Some ecological conditions may favor an indicator process, whereas others favor the Fisher process (e.g., ecological stability is thought to influence the strength of trait-preference genetic covariation in seaweed flies, Day and Gilburn 1997), but both processes may be compatibles. Between populations initial divergence in female preferences and male signal traits may be originated by a sensory drive or Fisher's runaway process, but the indirect-benefit selection model may be maintained in both populations. Thus, although chemical compounds are still indicators of male quality (CMI) in both populations, signals and preferences could have initially diverged as consequence of a Fisher's runaway process. Alternatively, different sources of direct selection on signals and/or preference could arise as adaptations to different physical environments following a 'sensory drive' process. It is difficult to disprove the proposal that the different male traits evolved, for example, to maximize signal conspicuousness in local environmental conditions (Endler 1992; Endler and Basolo 1998). Our results showed that males from 'Cueva' had higher proportions of certain steroids than males from 'Trigo' which might be related to different microclimatic conditions in the two populations. These steroids have high molecular weights, and may be good carrier compounds, which suggest a potential relationship between habitat characteristics and evolution of chemical signals (see also Martín and López 2006b). Many studies show that reproductive isolation may evolve because divergent selection may favor shifts in mate choice criteria onto traits that are most conspicuous in each environment (Endler, 1992; Boughman, 2001; Leal and Fleishman, 2004; Terai *et al.*, 2006). Differences in habitat characteristics may favor differences in sensory systems, which in turn may select for differences in signal design (Alberts, 1992; Endler, 1992). Inherent properties of signals, such as their chemical composition, affect signal conspicuousness, stability and probability of detection by males or females, so signals should evolve to get the maximum effectiveness (Alberts 1992; Endler 1992, 1993). The primary environmental factors that affect the spatial and temporal parameters of chemical signals in terrestrial environments are temperature, humidity and wind (Alberts 1992). High humidity and high temperatures increase evaporation and diffusion rates (Regnier and Goodwin 1977) and affect the persistence of chemical signals (McDonough et al. 1989). Volatility, a function of both molecular weight and chemical composition, determines in part how long a chemical signal remains active and dictates its pattern of spread in the environment, thus higher molecular weight results in lower volatility and a smaller diffusion coefficient (Alberts 1992). One strategy for increasing the life of a signal is the use of carrier compounds to retard evaporation of volatiles (Regnier and Goodwin 1977; Albone 1984). However, assortative preference in the present study is substantial in laboratory controlled conditions. This indicates that females of these different populations do not merely prefer male chemical signals more conspicuous in one particular environment. Moreover, the fact that preference and signal properties correlate with a measure of quality (CMI response) suggests that these are indica-

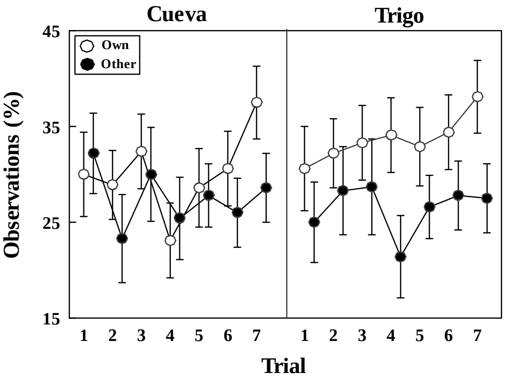

Fig. 4 Percent number of times (mean ± SE), during each of seven scent's choice trials, that female lizards *I. cyreni* from two close but isolated populations ('Cueva' and 'Trigo') were observed on paper strips scent marked by males of their own (open circles) or of a different population (black circles).

tors and not just different signals matched to different environments. The impact of other environmental components, such as diet, on variation in chemical signals cannot be ruled out a priori. Indeed, persuasive fits between diet and differences in chemical signals have already been documented in insects (Stennett and Etges, 1997; Liang and Silverman, 2000), where changes in chemical signals have been shown to affect dramatically species recognition and, in some cases, to result in premating isolation among lineages adapted to different foraging resources (Etges 1992; Etges and Ahrens 2001). The common lab housing strategy does not control for environmental effects, thus the conclusion of divergence between populations in signals is premature without the appropriate common garden experiment.

Recent molecular studies suggest that Iberian rock lizards comprise several closely related species and subspecies that occupy different isolated high mountain areas in the Iberian Peninsula (Mayer and Arribas 2003; Carranza et al. 2004), where they have evolved separately. However, it has paid little attention to the evolutionary causes that promoted their high diversification. We hope that our results will stimulate further research into the impact of chemical signals divergence on mate preferences and the formation of new species. Isolated but geographically close *I. cyreni* populations seem to constitute a good model to investigate the hypothesis of rapid population divergence led by sexual selection in a terrestrial vertebrate.

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Supplement 1 Principal components analysis for relative proportion of main chemicals in femoral gland secretions of male lizards *I. cyreni* of two close but isolated populations. Marked correlations are significant at *P*<0.001.

	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6
Cholesta-2,4-diene	0.31	-0.05	-0.14	**0.76**	0.07	-0.14
Cholesta-4,6-dien-3-ol	0.13	0.39	-0.06	-0.01	0.19	**-0.79**
Cholesta-3,5-diene	0.32	0.21	-0.01	-0.10	-0.02	**-0.74**
Cholesterol	**0.74**	0.04	0.21	0.10	-0.17	-0.35
Cholesta-5,7-dien-3-ol	**-0.54**	0.21	0.01	-0.11	0.01	0.33
Ergosta-5,22-dien-3-ol	-0.03	0.05	-0.06	-0.01	-0.55	0.08
Ergosterol	0.20	0.13	**-0.53**	0.37	-0.37	-0.38
Stigmasta-5,24(28)-dien-3-ol	0.54	0.03	-0.19	-0.28	**0.64**	-0.07
Campesterol	**0.74**	0.20	-0.39	0.01	0.20	0.21
Ergosta-5.7-dien-3-ol	-0.13	0.10	-0.52	-0.49	0.48	0.14
Lanost-8-en-3-ol	0.40	0.03	-0.17	0.09	0.12	-0.21
2,2-Dimethyl-cholest-8(14)-en-3-ol	-0.33	-0.21	-0.36	0.36	-0.32	0.21
γ-Sitosterol	**0.82**	-0.08	-0.16	0.13	0.02	0.11
24-Propylidene-cholest-5-en-3-ol	**0.61**	0.16	0.01	0.02	-0.11	-0.07
4,4-Dimethyl-cholest-7-en-3-ol	0.19	0.02	-0.04	**-0.60**	0.13	0.20
4,4-Dimethyl-cholesta-5,7-dien-3-ol	0.19	-0.07	**-0.71**	-0.34	0.24	0.05
Dodecanoic acid	0.14	0.23	0.01	0.19	-0.06	**-0.81**
Tetradecanoic acid	-0.07	0.06	0.18	0.20	-0.35	**-0.67**
Pentadecanoic acid	-0.07	0.26	0.12	0.49	**0.61**	0.12
Hexadecanoic acid	-0.11	**-0.83**	0.12	-0.04	0.13	0.18
Hexadecenoic acid	0.31	**-0.73**	-0.20	0.11	0.01	0.10
Hexadecanoic acid, ethyl ester	-0.15	-0.26	-0.15	-0.30	-0.16	-0.52
Heptadecanoic acid	0.01	-0.37	0.01	0.30	**0.60**	0.13
Octadecanoic acid	0.05	**-0.84**	0.34	-0.10	0.23	0.09
Octadecenoic acid	-0.21	**-0.85**	0.13	-0.18	-0.12	0.05
Octadecadienoic acid	-0.11	-0.40	**0.65**	-0.01	0.18	-0.39
Octadecenoic acid, methyl ester	0.14	0.24	0.15	**0.51**	-0.04	-0.15
Eicosanoic acid	0.36	-0.21	0.42	-0.15	-0.16	0.21
Eicosatetraenoic acid, methyl ester	-0.30	-0.09	**0.67**	0.02	0.38	0.21
Octadecanol	-0.22	0.40	0.12	-0.26	-0.31	0.47
Eicosanol	-0.15	0.22	0.11	**-0.79**	-0.32	0.01
Docosanol	**-0.57**	0.23	-0.09	-0.06	-0.21	0.50
Squalene	0.19	0.34	0.01	0.20	-0.49	-0.32
4-Hydroxy-hexadecanoic acid γ-lactone	0.03	0.24	0.11	**0.73**	-0.05	0.25
Eigenvalue	6.10	4.38	3.80	3.37	2.47	2.02
% Variance	17.95	12.89	11.19	9.94	7.28	5.95

ConclusioneS

1. El ambiente físico o social puede mediar la variación microgeográfica en la utilización de señales sexuales visuales o químicas entre poblaciones de lagartija carpetana (*Iberolacerta cyreni*). Características del microhábitat como una mayor cobertura de canchales rocosos, suelo desnudo o mayor distancia a los refugios, pueden promover el uso de señales químicas, mientras que una mayor cobertura de plantas puede promover una señalización más visual. Por otro lado, tanto la competencia intrasexual como la territorialidad pueden promover una mayor inversión en la señalización tanto química como visual ■

2. Los machos de lagartija serrana pueden compensar los efectos negativos de un incremento del riesgo de depredación debido a la coloración sexual conspicua modulando su comportamiento antidepredatorio. Las diferencias en la disponibilidad de refugios y en el número de depredadores potenciales, parecen promover diferencias entre especies en el compromiso entre comportamiento antidepredario y social. Así, los machos de *I. monticola*, pero no los de *I. cyreni*, compensan los efectos negativos de los ocelos azules laterales, que incrementan su conspicuidad visual. Además, los machos de *I. monticola* en mejor condición y con más ocelos azules laterales fueron más tímidos frente a los potenciales depredadores, mientras que los machos de *I. cyreni* en mejor condición y con más ocelos ventrales fueron más arriesgados. Esto sugiere que la regulación del comportamiento antidepredador puede también funcionar como un coste dependiente de la condición que promovería la señalización sexual costosa (honesta) ■

3. Los machos de lagartija cantábrica (*I. monticola*) con mayor "atractivo químico" para las hembras también muestran un mayor interés en el olor de una serpiente depredadora que utiliza estímulos químicos de las lagartijas para cazar. Esto sugiere una compensación individual del riesgo de depredación, mediada por la propia conspicuidad química. Esta compensación comportamental podría explicar la persistencia evolutiva de las señales químicas o del marcaje químico ■

4. Diferentes sistemas de apareamiento pueden determinar los beneficios directos y/o indirectos que los machos proveen a sus parejas y, por tanto, sesgar las preferencias de las hembras por diferentes rasgos de las señales de los machos. Así, en la especie más territorial (*I. monticola*) los rasgos de las señales químicas relacionados con la dominancia son preferidos por las hembras, mientras que en la especie menos territorial (*I. cyreni*) las hembras prefieren los rasgos de las señales químicas relacionados con la respuesta inmune de los machos. Ambas especies prefieren señales de los machos con mejor respuesta inmune, lo que sugiere que diferentes mecanismos pueden estar involucrados para asegurar la honestidad de las señales en cada especie ■

5. El aislamiento geográfico entre dos poblaciones de lagartija carpetana (*I. cyreni*) parece promover divergencias en las señales químicas de los machos y en las preferencias de las hembras, lo que podría llevar a un aislamiento reproductivo. Las hembras prefieren asociarse con las señales químicas de los machos de mejor calidad (medida como una mejor respuesta inmune), lo cual, sin embargo, está correlacionado con diferentes rasgos de las señales de los machos en cada población. Como resultado, las hembras se asocian preferentemente con los olores de los machos simpátricos, lo que podría promover el aislamiento sexual y la especiación ■