Universidad Autónoma de Tlaxcala

Posgrado en Ciencias Biológicas

Evolución de Rasgos Morfológicos y Conductuales en Libélulas y Caballitos del Diablo (Insecta: Odonata)

TESIS

QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTOR EN CIENCIAS BIOLÓGICAS

Presenta

Aldo Isaac Carrillo Muñoz

Director:

Dr. Martín Alejandro Serrano Meneses

Codirector:

Dr. Alejandro Ariel Ríos Chelén

Tlaxcala, Tlax. Octubre, 2020

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Tlaxcala, Tlax. Octubre, 2020

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PRESENTE

Los abajo firmantes, miembros del jurado evaluador del proyecto de tesis que **Aldo Isaac Carrillo Muñoz** realiza para la obtención del grado de **Doctor en Ciencias Biológicas**, expresamos que, habiendo revisado la versión final del documento de tesis, damos la aprobación para que ésta sea impresa y defendida en el examen correspondiente. El título que llevará es **"Evolución de rasgos morfológicos y conductuales en libélulas y caballitos del diablo (Insecta: *Odonata*)"**.

Sin otro particular, aprovechamos para enviarle un cordial saludo.

ATENTAMENTE
TLAXCALA, TLAX., OCTUBRE 22 DE 2020

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A quien corresponda:

Por medio de la presente, corroboro que la tesis del alumno **Aldo Isaac Carrillo Muñoz** fue sometida a programas anti-plagio conforme al reglamento de Bioética e Integridad Académica del Centro Tlaxcala de Biología de la Conducta (Universidad Autónoma de Tlaxcala). Cada uno de los capítulos fue revisado de manera independiente para comprobar su similitud con otros textos.

Primero, utilicé la plataforma https://my.plag.es para verificar cada capítulo. De los ocho archivos sometidos a escrutinio, siete de ellos no presentaron similitud con otros textos; uno de los capítulos ("Patterns of sexual dimorphism in flight agility in territorial and non-territorial Odonata") mostró similitud con un texto publicado. Este capítulo fue sometido a revisión utilizando la plataforma de https://www.paperrater.com/, la cual permite saber si el texto sometido a comprobación se trata de un artículo científico. Con esta plataforma comprobé que la información contenida en este capítulo no fue plagiada: la similitud fue alta con la versión publicada (artículo científico) del mismo capítulo (mismos autores).

En suma, con estas comparaciones se verifica que la tesis es un trabajo original. Anexo las evidencias de los programas anti-plagio utilizados.

Quedo a sus órdenes para cualquier aclaración o duda.

Atentamente,

Dr. Martín Alejandro Serrano Meneses
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Resumen

Los rasos biológicos, morfológicos (la forma, el tamaño o el color), conductuales (conductas específicas para tener acceso a los recursos energéticos o a las parejas potenciales), o moleculares (la expresión diferencial de ciertas proteínas o la secuencia específica de bases nitrogenadas de cada individuo) se encuentran bajo presiones selectivas naturales y sexuales. Los cambios que pueden generar estas presiones se pueden observar en diferentes niveles, en las diferencias entre sexos de una misma especie, entre individuos del mismo sexo, o los patrones de cambio evolutivo entre los distintos linajes de organismos.

Las libélulas y caballitos del diablo son un modelo de estudio ideal para entender las diferencias entre los organismos y la evolución de sus rasgos. Se puede obtener una gran cantidad de datos respecto a sus historias de vida y de la variación de sus rasgos biológicos. Estos organismos están asociados a cuerpos de agua, por lo cual es fácil encontrarlos. Generan gran cantidad de individuos en una sola temporada reproductiva, la cual suele durar pocos meses, dependiendo de cada especie o población. Por su tamaño pueden ser monitoreados en vida libre, ya que los adultos pueden ser marcados y seguidos de manera individual.

En esta tesis utilizo métodos filogenéticos comparativos y métodos estadísticos clásicos para poner a prueba las relaciones entre varias especies de estos insectos respecto a la variación de algunos de sus rasgos. De manera general, abordo las diferencias en las proporciones sexuales de los adultos y la diferencia en la agilidad de vuelo en todo el Orden, la selección sobre el tamaño corporal y su variación utilizando dos especies de caballitos del diablo territoriales, y además incluyo trabajos que tratan las historias de vida de algunas especies de estos insectos.

Podemos observar que los rasgos biológicos están fuertemente asociados a las estrategias reproductivas en estas especies de insectos, respondiendo a la intensidad de selección que presenta cada especie. Al integrar la variación filogenética observamos que la conducta sexual influye en los patrones evolutivos de cambio de sus proporciones sexuales y en la variación de la agilidad de vuelo. También podemos notar que el tamaño corporal se encuentra bajo presiones sexuales relacionadas a la capacidad de encontrar parejas potenciales a través del tiempo.

Índice

Evolución de Rasgos Morfológicos y Conductuales en Libélulas y Caballitos del Diablo (Insecta: Odonata)

Introducción

Actualmente sabemos que los organismos presentan modificaciones a través de las generaciones, es decir que las especies no son invariables (Darwin, 1859). Estas novedades evolutivas son trasmitidas de un ancestro o una pareja de ancestros hacia uno o varios descendientes por mecanismos de transferencia de información genética, es decir a través de la herencia biológica (Fisher, 1930; Dobzhansky, 1973). Aunado a esto, la manera en que cada organismo se enfrenta a su ambiente, permite una plasticidad morfo-fisiológica, generando las formas, tamaños y colores que podemos observar en cada especie (Darwin, 1869, 1871; Huxley, 1942; Dobzhansky, 1973; Pigliucci et al., 2006).

Muchas de las características particulares de los organismos, como la forma, el tamaño, los colores en diferentes partes del cuerpo y las tasas metabólicas, así como también ciertas conductas, entre muchas otras particularidades, pueden ser heredables a las siguientes generaciones. Estas características heredables las podemos englobar en lo que llamamos "rasgos" o "caracteres biológicos", los cuales pueden ser morfológicos, como la longitud corporal total o la forma de cierta parte del cuerpo; moleculares, como la expresión de una proteína; o conductuales, como los comportamientos expresados por algunos machos que defienden sus recursos de otros machos rivales; o alguna combinación de estos en un cierto punto del desarrollo de los organismos, como el tamaño al nacimiento o a la madurez sexual, la proporción de machos y hembras en una población, la edad y el tamaño cuando mueren, entre otros (Stearns, 1992). Las relaciones entre estos rasgos biológicos y las historias de vida de los organismos han sido de gran interés entre biólogos evolutivos y ecologistas, como los son

Charles Darwin y Alfred Russel Wallace (Ruiz-Gutiérrez y Rodríguez-Caso, 2013). El estudio de estos rasgos, sus variaciones y la relaciones con otros rasgos y el ambiente ha permitido que podamos entender diversos patrones de cambio a gran escala, como es la propuesta de Carl Bergmann (1847) en la que se plantea que en un grupo taxonómico de amplia distribución, los organismos de poblaciones que se distribuyan en climas fríos serán más grandes que los que se distribuyen en zonas cálidas (regla de Bergman); o la propuesta de Edward Drinker Cope (1887, 1896; ver Stanley, 1973) con relación al tamaño de los organismos, la cual menciona que los linajes animales tienden a aumentar de tamaño en un tiempo evolutivo determinado (regla de Cope); o la propuesta de Bernard Rensch (1950), la cual trata sobre la diferencia de tamaño entre los sexos y como el tamaño aumenta o disminuye en relación al tamaño del sexo más grande (regla de Rensch). Como estas propuestas existen otras más que tratan de dar explicación a los patrones de cambio de los rasgos en los organismos.

Son las fuerzas selectivas (selección natural y selección sexual) las que se encargan de moldear a los rasgos, dependiendo de las interacciones entre individuos de la misma especie (entre individuos del mismo sexo y entre sexos), con las demás especies y con el ambiente (Stearns, 1992). Además, los distintos estados de carácter, es decir las distintas formas, tamaños, colores, proporciones o estrategias, de cada rasgo biológico, reflejan parte de la historia evolutiva y la adaptación que cada linaje ha tenido con su entorno a lo largo del tiempo (Stearns, 1992).

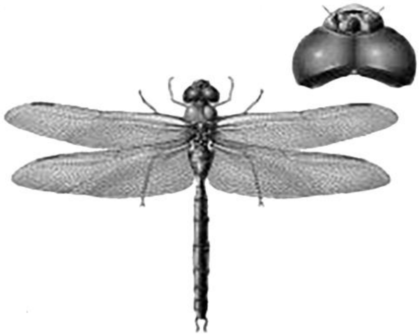
El enfoque moderno para el estudio de la evolución de los rasgos biológicos de los organismos y de sus historias de vida incluye una amplia gama de herramientas. Algunas de ellas son: la taxonomía y la sistemática, que se encargan de asignar las categorías jerárquicas de cada grupo biológico respecto a sus características morfológicas y/o genéticos; la etología, que estudia el comportamiento de los organismos y su relación con otros individuos y el ambiente, y la ecología, que trata de entender las relaciones de los organismos con su ambiente. Todas ellas nos proveen de información útil para entender las historias de vida y evolutivas de cada linaje. Además, la inclusión de técnicas matemáticas para relacionar la variación de los rasgos con su adecuación a diferentes escalas nos permite entender una gran cantidad de fenómenos biológicos y como estos han evolucionado (Cuesta, 2009). Estas relaciones se pueden modelar enfocándose en una sola población y en un tiempo corto (e.g. una temporada reproductiva), a lo largo de la vida completa de los organismos de interés (e.g. éxito de apareamiento vitalicio) o a escalas de tiempo más grandes, como es la evolución de ciertos rasgos en todo un linaje utilizando las relaciones de ancestría-descendencia o filogenéticas de dicho linaje (Soler, 2002).

Libélulas y caballitos del diablo como organismos de estudio

Las libélulas (Anisoptera) y los caballitos del diablo (Zygoptera) son insectos que pertenecen al orden Odonata. Los odonatos, junto con sus parientes cercanamente relacionados, pero ya extintos, los Odonatoptera, fueron de los primeros insectos en levantar el vuelo hace más de 320 millones de años en la era del Carbonífero, en ese entonces existían organismos que llegaban a medir hasta 70 cm de anchura entre los extremos de sus alas (*Meganeuropsis permiana*) (Suhling et al., 2015). El número de especies descritos para este orden de insectos es alrededor de 5600 especies vivas y 600 fósiles (Beutel et al., 2014). Las principales diferencias entre estos dos subórdenes de Odonata las podemos ver en el Cuadro1.

El desarrollo de estos insectos depende de cuerpos acuáticos. Los huevos de la mayoría de las especies son depositados en el agua, en plantas o en substratos húmedos. Cuando los huevos eclosionan, surgen las larvas o ninfas, estas cazan pequeñas presas e incluso a otros miembros de su misma especie. Los organismos de la mayoría de las especies pasan uno o dos años en el estado de ninfa acuática, sin embargo, existen algunas especies que llegan a pasar ocho años en este estado. Al alcanzar cierto grado de desarrollo, las larvas sufren su última ecdisis (cambio de exoesqueleto para crecer) para emerger como adultos voladores. Estos adultos recién emergidos o tenerales pasan algunas horas o hasta algunos días a la espera de que se endurezca su nuevo exosqueleto. Cuando su exoesqueleto se encuentra totalmente endurecido, su tamaño corporal queda fijo, así como el tamaño y coloración de la pigmentación alar que se presenta en algunas especies y que se pueden considerar adultos sexualmente maduros. Una vez que los adultos han madurado sexualmente, buscan organismos del sexo contrario para reproducirse (Corbet, 1999; Suhling et al., 2015). En la Figura 1 podemos ver un esquema del ciclo de vida de una libélula.

Cuadro 1. Principales diferencias entre libélulas (Anisoptera) y caballitos del diablo (Zygoptera). Algunas de estas diferencias no se cumplen para todas las especies.

Suborden	**Anisoptera**	**Zygoptera**
Nombre común	Libélula	Caballito del diablo
Etimología griega	anisos=desigual, pteron= ala	zugós=igual, pterá=ala
Significado	Alas desiguales	Alas iguales
Imagen		
(Imagenes modificadas de: “Morphological differences between Anisoptera and Zygoptera”, https://medicalart.johnshopkins.edu/)		
Alas	Diferentes entre las anteriores y posteriores	Iguales entre las anteriores y posteriores
	Ensanchadas en la base	Estrechas en la base
	Se mantienen abiertas cuando están perchadas	Se mantienen cerradas cuando están perchadas
Ojos	Juntan al medio de la cabeza	Separados
	Cubren la mayoría la cabeza	Se encuentran a los lados de la cabeza

Cuerpo	Robusto	Delgado

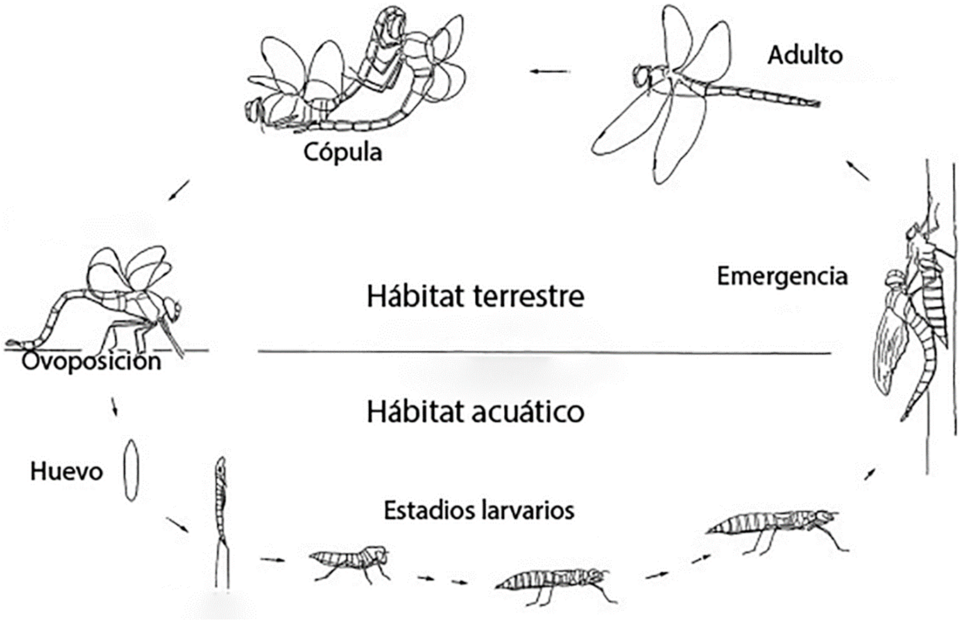


Figura 1. Ciclo de vida de los odonatos. El ciclo empieza con la oviposición de los huevos en hábitats acuáticos, de este huevo eclosionan las larvas y después de varios estadios en los cuales crecen los organismos emergen los adultos al ambiente terrestre, los adultos se aparean y forman la cópula para fertilizar nuevos huevos que serán depositados en hábitats acuáticos. Modificado de Suhling et al., 2015.

Estos insectos poseen características corporales y de comportamiento muy interesantes y vistosas que los hace sujetos de una gran cantidad de investigaciones en diversos ámbitos de la ciencia, desde la biología más básica, como es la descripción taxonómica y sistemática de cada especie, pasando por investigaciones en ecología, fisiología y conservación, entre otras disciplinas, hasta estudios enfocados en los patrones de cambio evolutivos de sus rasgos (Córdoba-Aguilar, 2008). Además, ya se ha secuenciado una cantidad considerable de información molecular de diferentes especies de Odonata (e.g. Bybee et al., 2008; Carle et al., 2008; Dumont et al., 2010; Dijkstra et al., 2014; Letsch et al., 2016), asimismo se han generado una buena cantidad de conocimiento sobre las historias de vida, tanto de estudios ecológicos, como fisiológicos y conductuales (Corbet, 1999; Córdoba-Aguilar, 2008). Por ejemplo, sabemos que sus tamaños corporales quedan fijos al alcanzar la madurez sexual (Anholt et al., 1991; Fincke et al., 1997; Serrano-Meneses et al., 2007). Los adultos regresan a los cuerpos de agua en busca de parejas para reproducirse y ya que sus tallas son relativamente grandes, los hace organismos manejables y rastreables en condiciones de vida libre y en laboratorios (Cordero-Rivera y Stoks, 2008). Algunas otras características interesantes que podemos observar ente los adultos en este grupo son su patrón de dimorfismo sexual en tamaño, pasando de hembras más grandes que los machos, hembras y machos del mismo tamaño y machos más grandes que las hembras (Serrano-Meneses et al. 2008a); poseen sistemas de apareamiento alternativo, ya sea que los machos defiendan territorios contra otros machos para la obtención de parejas a través de contiendas violentas, o señalizando sus capacidades de mantener un territorio mediante coloraciones en sus alas, o que no lo hagan y busque a sus parejas de modo activo a lo largo de las orillas de los cuerpos de agua (Corbet, 1999; Suhonen et al., 2008). Se ha observado que la pigmentación alar que se presenta en algunas especies, funciona para la atracción de parejas o para la advertencia de su calidad (Corbet, 1999; Serrano-Meneses et al. 2008b).

Presentación

Así, presento una variedad de trabajos en los que investigamos (ver los autores de cada capítulo) las relaciones entre diversos rasgos, morfológicos, moleculares y conductuales, para entender como las fuerzas selectivas (selección natural y selección sexual) se involucran para mantenerlos o modificarlos, utilizando a las libélulas y a los caballitos del diablo (Odonata) como organismos de estudio. Los primeros dos trabajos tratan con la evolución de diferencias sexuales y con los sistemas de apareamiento en las especies de todo el orden Odonata. El primero de ellos (“The evolution of adult sex ratios in dragonflies and damselflies (Insecta: Odonanta)”) relaciona las diferencias entre el número de individuos de cada sexo en las diferentes especies, con rasgos como el tamaño corporal, la diferencia de tamaño entre los sexos y la estrategia reproductiva de los machos. El segundo (“The evolution of sexual dimorphism in flight agility in territorial and not territorial Odonata”), relaciona la agilidad de vuelo y la diferencia de esta agilidad entre machos y hembras con ambos subórdenes de Odonata, Anisoptera y Zygoptera. En el tercero y cuarto trabajo que presento, el tamaño corporal es el rasgo morfológico que se encuentra bajo escrutinio para conocer las relaciones de este rasgo entre los sexos, con su éxito de apareamiento, supervivencia y con otros rasgos de tamaño y/o color en las alas. En ellos se evalúan las relaciones de tamaño corporal de dos especies de una familia de Odonata, el caballito del diablo escarlata americano: *Hetaerina americana*, y el caballito escarlata de montaña: *H. vulnerata*, respectivamente. Las especies de esta familia presenta características bastante útiles para entender cómo actúan las fuerzas selectivas sobre los individuos. El tercer trabajo ("Assortative mating by size in the American rubyspot damselfly (*Hetaerina americana*)") trata con la manera de cómo se seleccionan las parejas reproductivas con respecto al tamaño de los individuos. El cuarto trabajo ("Seasonal variation in the allometry of the pigmentation of the wings in adult males of the territorial devil's horse *Hetaerina vulnerata* (Insecta: Odonata)") trata sobre la variación del tamaño y área de la pigmentación alar a lo largo de una temporada reproductiva. Estos cuatro trabajos presentan una perspectiva de la evolución del tamaño corporal en Odonata y como se relaciona con otros rasgos de tamaño y comportamiento, revelando el papel de la selección sexual como una fuerza selectiva activa para mantener o modificar los rasgos de tamaño entre individuos de un mismo sexo y entre ambos sexos en una sola especie. Además, presento dos trabajos que abordan la historia natural de algunas especies de este Orden de insectos, la primera como un artículo de divulgación sobre el trabajo que se desarrolla en el laboratorio donde llevé a cabo mis estudios de doctorado ("El caballito escarlata de montaña y la coloración animal"). El segundo, una nota científica sobre la fauna encontrada en una región de alta prioridad para su conservación en México ("New records from the Chimalapas-Uxpanapa region, Mexico (Odonata: Calopterygidae, Heteragrionidae, Polythoridae, Thaumatoneuridae, Coenagrionidae, Gomphidae, Libellulidae)").

Literatura citada

Anholt, B.R. (1991). Measuring selection on a population of damselflies with a manipulated phenotype. Evolution, 45, 1091-1106. https://doi.org/10.1111/j.1558-5646.1991.tb04377.x

Bergmann, C. (1847). Uber die Verhaltnisse der warmeokonomie der Thiere zu uber Grosso. Gottinger studien, 3, 595-708. https://archive.org/details/bub_gb_EHo-AAAAcAAJ/page/n4/mode/2up

Beutel, R., Friedrich, F., Ge, S.Q. y Yang, X.K. (2013). Insect morphology and phylogeny. Boston, USA. Walter De Gruyer.

Bybee, S.M., Ogden, T.H., Branhama, M.A. y Whiting, M.F. (2008). Molecules, morphology and fossils: a comprehensive approach to odonate phylogeny and the evolution of the odonate wing. Cladistics, 24, 477-514. https://doi.org/10.1111/j.1096-0031.2007.00191.x

Carle, F.L., Kjer, K.M. y May, M.L. (2008). Evolution of Odonata, with special reference to Coenagronoidea (Zygoptera). Arthropod Systematic and Phylogeny, 66, 37-44.

Cope, E.D. (1887). The origin of the fittest. New York, USA. D. Appleton & Co.

Cope, E.D. (1896). The primary factors of organic evolution. Chicago, USA. Open Court Publ. Co.

Corbet, P.S. (1999). Dragonflies: behaviour and ecology of Odonata. Colchester, UK. Harley books.

Córdoba-Aguilar, A. (2008). Dragonflies and damselflies: model organisms for ecological and evolutionary research. Oxford, UK. Oxford University Press.

Cuesta, J.A. (2009). Las matemáticas de la evolución. La Gaceta de la RSME, 12, 667-686.

Darwin, C. (1859). On the origin of species. London, UK. John Murray.

Darwin, C. (1871). The descent of man, and selection in relation to sex. London, UK. John Murray.

Dijkstra, K.D.B., Kalkman, V.J., Dow, R.A., Strokvis, F.R. y Van-Tol, J.A.N. (2014). Redefining the damselfly families: a comprehensive molecular phylogeny of Zygoptera (Odonata). Systematics Entomology, 39, 68-96. https://doi.org/10.1111/syen.12035

Dobzhansky T. (1937). Genetics and the origin of species. New York, USA. Columbia University Press.

Dumont, H.J., Vierstraete, A. y Vanfletren, J.A. (2010). A molecular phylogeny of the Odonata (Insecta). Systematic Entomology, 35, 6-18. https://doi.org/10.1111/j.1365-3113.2009.00489.x

Fincke, O.M., Waage, J.K., y Koenig, W.D. (1997). Natural and sexual selection components of odonate mating patterns. En: The evolution of mating systems in insects and arachnids (J.C. Choe y B.J. Crespu, eds.), pp. 58-74. Cambridge, UK. Cambridge University Press. doi:10.1017/CBO9780511721946.004

Fisher, R.A. (1930). The genetical theory of Natural Selection. Oxford, UK. Clarendon Press.

Huxley, J. (1942). Evolution. The modern synthesis. London, UK. George Alien & Unwin Ltd. https://archive.org/details/in.ernet.dli.2015.218848/mode/2up

Letsch, H; Gottsberg, B. y Ware, J.L. (2016). Not going with the flow: a comprehensive time calibrated phylogeny of dragonflies (Anisoptera: Odonata: Insecta) provides evidence for the role of lentic habitats on diversification. Molecular Ecology, 25, 1365-2940. https://doi.org/10.1111/mec.13562

Pigliucci, M., Murren, C.J. y Schlichting, C.D. (2006). Phenotypic plasticity and evolution by genetic assimilation. The Journal of experimental biology, 209, 2362-2367. doi:10.1242/jeb.02070

Preston, S.D., Kringelbach, M.L., y Knutson, B. (2014). The interdisciplinary science of consumption. Cambridge, UK. The MIT Press.

Rensch, B. (1950). Die Abhängigkeit der relativen Sexualdifferenz von der Körpergrösse. Bonner Zoologische Beiträge, 1, 58-69.

Roff, D. (2002). Life History Evolution. Sunderland, USA. Sinauer Associates, Inc.

Ruiz-Gutierrez, R. y Rodriguez-Caso, J.M. (2014). Alfred Russel Wallace y Charles Darwin: dos maneras de interpretar la evolución. OIKOS, 10, 4-6.

Serrano-Meneses, M.A; Córdoba-Aguilar, A., Azpilicueta-Amorín, M., González-Soriano, E. y Székely, T. (2008a). Sexual selection, sexual size dimorphism and Rensch's rule in Odonata. Journal of Evolutionary Biology, 21, 1259-1273. https://doi.org/10.1111/j.1420-9101.2008.01567.x

Serrano-Meneses, M.A; Sánchez-Rojas, G. y Córdoba-Aguilar, A. (2008b). Sexual selection as the possible underlying force in calopterygid wing pigmentation: comparative evidence with *Hetaerina* and *Calopteryx* (Zygoptera: Calopterygidae). Odonatologica, 37, 221-233. http://natuurtijdschriften.nl/download?type=document;docid=592631

Serrano-Meneses, M.A., Córdoba-Aguilar, A., Méndez, V., Layen, S.J. y Székely, T. (2007). Sexual size dimorphism in the American rubyspot: male body size predicts male competition and mating success. Animal Behaviour, 73, 897-997. https://doi.org/10.1016/j.anbehav.2006.08.012

Cordero-Rivera, A. y Stocks, R. (2008). Mark–recapture studies and demography. En: Dragonflies and damselflies. Model organisms for ecological and evolutionary research (A. Córdoba-Aguilar, ed.), pp. 7-20. Oxford, UK. Oxford University Press.

Soler-Cruz, M. (2002). ¿A qué nivel actúa la selección natural? En: Evolución: la base de la biología (M. Soler-Cruz, ed.), pp. 75-82. España. Proyecto Sur.

Stanley, S.M. (1973). An explanation for Cope's rule. Evolution, 27, 1-26. https://doi.org/10.1111/j.1558-5646.1973.tb05912.x

Stearns, S. (1992). The evolution of life histories. Oxford, UK. Oxford University Press.

Suhling, F., Sahlén, G., Gorb, S., Kalkman, V.J., Dijkstra, K.-D.B., & van Tol, J. (2015). Order Odonata. En: Thorp and Covich's Freshwater Invertebrates: ecology and general biology (J.H. Thorp y D.C. Rogers, eds.), pp. 893–932. Amsterdam, DE. Academic Press. https://doi.org/10.1016/b978-0-12-385026-3.00035-8

Suhonen, J. Rantala, M.J. y Honkavaara, J. (2008). Territoriality in Odonata. En: Dragonflies and damselflies. Model organisms for ecological and evolutionary research (A. Córdoba-Aguilar, ed.), pp. 7-20. Oxford, UK. Oxford University Press.

Objetivos

Objetivo general

Poner a prueba las relaciones entre diversos caracteres morfológicos, conductuales y moleculares para conocer la influencia de la selección natural y sexual sobre la evolución de los rasgos biológicos en libélulas y caballitos del diablo.

Objetivos particulares

- Comprender cómo afecta la variación del tamaño corporal de los machos y de las hembras, la variación de la pigmentación alar y la táctica reproductiva de los machos, en las tendencias evolutivas de cambio de las proporciones sexuales de los adultos de Odonata.
- Comprender la relación entre las tácticas reproductivas de cada especie con las diferencias sexuales de la agilidad de vuelo y su evolución en Odonata.
- Conocer si existen apareamientos selectivos por tamaño en una especie de caballito del diablo (*Hetaerina americana*).
- Entender la relación entre el tamaño corporal y la pigmentación a alar de los machos durante una temporada reproductiva completa, en una especie de caballito del diablo (*Hetaerina vulnerata*).
- Exponer el estudio de la evolución del color utilizando el modelo de estudio de los insectos del Orden Odonata.
- Mostrar algunas especies del Orden Odonata de nuestro país, además de exponer la necesidad de continuar con el estudio de biodiversidad en este Orden de insectos.

Cada uno de los capítulos presentados en esta tesis aborda cada objetivo particular.

The evolution of adult sex ratios in dragonflies and damselflies (Insecta: Odonanta)

Aldo Isaac CARRILLO-MUÑOZ, Adolfo CORDERO-RIVERA, Araxi URRUTIA, Martín Alejandro SERRANO-MENESES y Tamás SZÉKELY. (2020).

Manuscrito

The evolution of adult sex ratios in dragonflies and damselflies (insecta: odonata)

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Abstract

The proportions of males and females has determinant effects on behaviour and mating systems. Deviations from a 50:50 sex ratio is common in animals, derived from sexual differences in survival and/or mortality probabilities, migration rates and maturation times at different development stages. Generally, sexual differences in morphology and behaviour are linked to population dynamics that affects sex ratios. The interest in the variation of sex ratios in Odonata dates, at least, from the nineteen century, but its evolutionary understanding is still not completely understood. First, we construct a molecular phylogeny including 99 Odonata species, of which we collect sex ratio at emergence as adults, at sexual maturity, body size, male wing pigmentation and mating system. Then, we use phylogenetic comparative methods to modelled morphological and behavioural traits to understand the evolutionary trends of the adult sex ratio (ASR). All the families with ASR estimated at emergence (exuviae picking) do not show any sexual bias, but at sexual maturity (mark-recapture method), most of them are male biased. The mating system of each species, is the one of the drivers that maintain or modify the ASR in Odonata at an evolutionary level, but depend on the portion population taking account, newly emerged or sexually mature adults, that morphological traits interact with them. In the former, the body size and wing pigmentation are evolutionary related to ASR differently between non-territorial and territorial species. Meanwhile, in the latter, sexual size dimorphism is evolutionary related with ASR differently between non-territorial and territorial species.

Keywords: Adult sex ratio, Odonata, Mating system, Body size, Exuviae, Recapture.

“I formerly thought that when a tendency to produce the two sexes in equal numbers was advantageous to the species, it would follow from natural selection, but I now see that the whole problem is so intricate that it is safer to leave its solution for the future” (Darwin, 1871).

Introduction

The adult sex ratio (ASR), the proportion of adult males in an adult population, is an important property of breeding populations (Bessa-Gomes et al., 2004; Le Galliard et al., 2005; Székely et al., 2014), which has determinant effects on the ecology, behaviour, population dynamics and breeding systems of the species (Kokko & Jennions, 2008; Székely et al., 2014). Darwin (1871) realised that the proportions of the sexes in animals (e.g. birds, fish, insects, mammals) were not equal. Such differences were so wide ranging and varied, that he was unable to formulate an explanation for them at the time. Indeed, deviations from a 50:50 sex ratio is common in both, population and species levels in many animals (Bessa-Gomes et al., 2004, Székely et al., 2014). Amongst vertebrates, for instance, many birds exhibit male-biased ASRs (Carmona-Isunza et al., 2017), whereas mammals tend to exhibit female-biased ASRs (Solberg et al., 2002). Biased ASRs have also been observed in several invertebrates (Poulin, 1997a, b), and there are extreme documented cases in which males are almost entirely absent (Dyson & Hurst, 2004), or even fully absent from populations (Normark, 2014).

Biases in ASRs can result from different factors affecting individuals at different stages of their lives (reviewed by Székely et al., 2014): (i) biased primary sex ratios, (ii) sex differential mortalities of young and adults, (iii) differences in maturation times between males and females, and (iv) sex-specific dispersal and migration patterns (Wilson, 1975; Bessa-Gomes et al., 2004; Veran & Beissinger, 2009). In many organisms, sex ratios are already biased at conception or birth, which can be adaptive if the cost of providing parental care differs according to the sex of the offspring (Clutton-Brock, 1991; West, 2009). In animals with environmental sex determination, hatchling sex ratios may be biased in response to temperature, as it occurs in many fishes and reptiles (Pen et al., 2010). Secondary sex ratios may be further shaped by sex-differential mortality of juveniles, which may arise from viability selection acting, for instance, on larger-than-average individuals (Blanckenhorn, 2000). Besides, since maturation times tend to differ between males and females in many taxa, biases in sex ratios may be observed in populations (e.g. Osmundson, 2006; Hirst et al., 2010; Kusano & Inoue, 2011), mainly through the recruitment of those individuals maturing earlier (Donald, 2007). Also, sex-specific migratory patterns can skew the local proportions of males to females to extreme values (Hamilton, 1967; Casale et al., 2005), although these may be balanced out at a metapopulation level, since those individuals leaving a population will move to a different one if they remain alive (Székely et al., 2014). Finally, the ASR may be different depending on which adults are taken into account, if all the adults are considered (ASR) or if only the sexually matured (OSR: operational sex ratio), as this two sex ratio can reflect different selective pressures and have different implications for populations (Kvarnemo & Ahnesjo, 1996; Veran et al., 2009; Székely et al., 2014; Carmona-Isunza et al., 2017).

Males and females often exhibit differences in morphology and behaviour (Arnqvist & Rowe, 2005; Fairbairn et al., 2007). The evolution of dimorphic traits can lead to differences in energy consumption, foraging ecology, and sex-specific mortalities, which are in turn likely to influence ASR (Székely et al., 2014). For instance, in food-limited environments, large body size and male-biased sexual size dimorphism (SSD) correlate with lower male survival in ungulates (Toïgo & Gaillard, 2003). In birds, SSD co-varies positively with higher sex-specific mortality patterns in juvenile stages, with the highest mortality being experienced by the sex with the largest body size (Kalmbach & Benito, 2007). Similarly, sexually selected traits are also related to lower male survival rates, as it has been observed in guppies and barn swallows (Moller & Szép, 2002; Godin & McDonough, 2003). Finally, ASR can have an important influence on the mating system of a given species, but through complex feedbacks, mating systems may also influence ASR (Székely et al., 2014; Clutton-Brock, 2017). For instance, polygamy may impose sex-specific costs, whilst biases in ASR promote unequal mating opportunities, thereby promoting polygamous mating systems (Eberhart-Phillips et al., 2017).

Dragonflies (Anisoptera) and damselflies (Zygoptera) (Insecta: Odonata), one the earliest branching groups of insects (Wheeler et al., 2001) are an ideal model to study the potential causes of ASR variation. Biased ASR have been reported in many odonates for over a century (e.g. Tillyard, 1917). Darwin (1871) reported male-biased ASR in two Odonata genres: Gomphus and Hetaerina, with males outnumbering females 4:1. Many odonate species exhibits marked between-species differences in ASR, ranging from strongly male-biased species (e.g. Somatochlora alpestris, Knaus, 1999) to species with female-only populations (e.g. Ischnura hastata, Cordero-Rivera et al., 2005). Also, odonate species exhibit a range of body sizes and SSD, which spans from female-biased to male-biased SSD; in territorial species, female-biased SSD increases with male agility, whilst male-biased SSD increases when lower levels of male agility evolve (Serrano-Meneses et al., 2008a, b). In several species, males exhibit wing pigmentation (WP), a trait that has been commonly linked to sexual selection (e.g. Córdoba-Aguilar & Cordero-Rivera, 2005), although the majority of studies linking this trait to sexual selection have been carried out in species within the Calopterygidae family (e.g. Calopteryx and Hetaerina genres). There is also a lack of comprehensive macro-ecological comparative studies testing this link (see Svensson & Waller, 2013, for a study focusing on calopterygid species). In any case, WP is known entail costs for males. In H. americana, for instance, the experimental enlargement of male WP is correlated with a decrease in survival (Grether, 1997). In species with distinct male morphs, in which males occur as either pigmented or clear-winged, it is the former that experience the lowest survival rates (e.g. Mnais pruinosa costalis; Tsubaki et al., 1997). Finally, two main mating systems have been described and commonly used in ecological and evolutionary studies of Odonata: territoriality, in which males defend a territory to which females are attracted for copulations (resource defence polygyny), and non-territoriality, in which males do not establish or defend a territory, and instead actively look for females (non-resource defence polygyny) (Conrad & Pritchard, 1992). Sexual selection is commonly more intense in territorial species, compared to non-territorial ones (Suhonen et al., 2008).

A number of studies have investigated ASR and in Odonata, using different methodologies. For instance, in an optimisation analysis of sexually dimorphic life histories, Crowley & Johansson (2002) predicted that males should be larger than females at emergence, and that sex ratio should be predominantly female-biased in territorial species, compared to non-territorial ones. Johansson et al. (2005) later used a phylogenetic comparative analysis to investigate the relationship between SSD and ASR in Odonata, and their phylogenetic correction suggested that both variables are unrelated. However, the sample sizes used by Johansson et al. (2005) are limited (21 species), and the relationship between ASR and mating systems was not tested. Also, to account for the general male-biased ASR observed in Odonata, Cordero-Rivera & Stoks (2008) put forward five hypotheses, based mainly on differences in existing biases at emergence, maturation times, and high female mortality. Nonetheless, Cordero-Rivera & Stoks (2008) suggest that some of these hypotheses, on their own, are insufficient to cause the pattern, and critically, the hypotheses proposed were not tested. Thus, considering the shortfalls of previous studies, here we investigated potential explanations for ASR variation in Odonata using phylogenetic comparative methods. Specifically, we investigated the relationship between ASR and (i) body size (BS), (ii) sexual size dimorphism (SSD), (iii) male wing pigmentation (WP), and (iv) mating systems (MS). We initially aimed to test the association between ASR and other variables (e.g. sex-specific survival) but the data was not readily available in the literature for the vast majority of the species used in this study. Taken together, our analyses are the most comprehensive tests of ASR in Odonata to date.

Methods

Species selection

A species were included in our study if (i) data on ASR were available for at least one population per species, and (ii) there were molecular data for at least two genes (one nuclear and one mitochondrial; see below), in order to construct a phylogenetic hypothesis to correct for the phylogenetic non-independence of data.

ASR data compilation

ASR data were compiled from a total of 99 species of both Odonata suborders, Anisoptera (n = 59) and Zygoptera (n = 40). Data was primarily collected from Cordero-Rivera & Stoks (2008) chapter as well as from additional sources available in the literature (see Supplementary Material 1 for a full list of species and source references). Where ASR estimates were available for multiple populations of one species, all available estimates were averaged. ASR is expressed as the proportion of males in the adult population. In literature, ASR was obtained either from exuviae picking (n = 62 species), or from mark-recapture studies (n = 47 species), and since the ASR estimates differ between methods (see Results), the data were analysed separately.

Morphological and behavioural data compilation

Data on morphological and behavioural traits were collected from literature and specialised databases for the species with available ASR data. BS for each species was measured as the average of the log_{10} transformed values of body length (in mm) of males and females. The SSD was calculated as log_{10}(male body length) - log_{10}(female body length) (Lovich & Gibbons, 1992). The use of both, male body length as a proxy of BS, and the manner in which we quantified SSD, have been used in previous evolutionary studies of Odonata (e.g. Serrano-Meneses et al., 2008a, c). Male WP was quantified as the average proportion of pigment covering the surface of an anterior and a posterior wing, and was calculated from at least three photos from different sources, per male, per species, whenever available. Data on MS were collected from multiple sources. Thus, species were classified by their MS as non-territorial and territorial (Corbet, 1999; Suhonen et al., 2008), and coded as 0 and 1 prior to analyses, respectively. All data are available in Supplementary Material 1.

Phylogeny

In order to correct for phylogenetic inertia (Harvey & Pagel, 1991), we constructed a molecular phylogeny comprising 99 Odonata species, including four other insect taxa as outgroup (Blattodea: *Blattella germanica*; Coleoptera: *Sitophilus zeamais*; Ephemeroptera: *Callibaetis ferrugineus*: Orthoptera: *Oxya chinensis*). Four mitochondrial and three ribosomal nuclear genes commonly used for phylogenetic reconstructions (e.g. Bybee et al., 2008; Carle et al., 2008; Dumont et al., 2010; Dijkstra et al., 2014; Letsch et al., 2016), and widely available in Odonata species were selected. Thus, we obtained the DNA sequences of the mitochondrial genes (i) 12S rRNA (12S), (ii) tRNA-Valine (Val), (iii) 16S rRNA (16S), and (iv) cytochrome c oxidase subunit I (COI); and the sequences of the ribosomal nuclear genes (v) 18S rRNA (18S), (vi) 5.8S rRNA (5.8S), and (vi) 28S rRNA (28S), from GenBank (NCBI Resource Coordinators, 2018). The full list of the GenBank accession numbers for each gene is provided in the Supplementary Material 2. The sequences for each gene were then independently aligned using the MUSCLE algorithm (Robert, 2004), as implemented in PhyDE (ver. 9971; Müller et al., 2012). The aligned sequences were later edited to remove any positions not included in every sequence (12S = 693 bp, Val = 76 bp, 16S = 1263 bp, and COI = 668 bp for mitochondrial genes; 18S = 1969 bp, 5.8S=163 bp, and 28S = 4153 bp for nuclear genes). jModelTest (ver. 2.1.10; Guindon & Gascuel, 2003; Darriba et al., 2012) was used to estimate the best-fit nucleotide change rate model of each gene, which was selected according to its AICc value, parameter numbers and -lnLilkelihood. The following models were selected for each gene: 12S = HKY+I+G, Val = HKY+G, 16S = HKY+I+G, COI = HKY+I+G for mitochondrial genes, and 18S = SYM+I+G, 5.8S = JC+I, 28S = GTR+I+G for nuclear genes.

Curated alignments of the sequences of the 99 in-group Odonata species and the four outgroup species for the seven genes were concatenated into a single sequence using Mesquite (ver. 3.51.; Maddison & Maddison, 2018). This resulted in an alignment of 8,985 bp in length. This concatenated alignment and nucleotide change rate models for each gene were used to construct a posterior probability consensus tree using MrBayes (ver. 3.2; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; Altekar et al., 2004). The analysis consisted of two independent runs, each with 10,000,000 generations and four chains, sampling each 1,000 generations. The initial 25% of samples were discarded (burnin) in order to eliminate non-converging samples. The effective sampling size and parameter convergence was assessed with Tracer (ver. 1.7; Rambaut et al., 2018) for each run before mixing. Outgroup species were pruned from the resulting consensus tree using the R (R Core Team, 2019) package ape (Paradis et al., 2004). Our posterior probability consensus tree (Fig. 1) is concordant with previous published phylogenies of Odonata for the sets of overlapping species (e.g. Bybee et al., 2008; Carle et al., 2008; Dumont et al., 2010; Dijkstra et al., 2014).

Comparative analyses

To face the problem of lack of phylogenetic independence, the phylogenetic comparative methods seek correlations with the appearance of the traits of interest and life histories between two or more species under a phylogenetic hypothesis of ancestry-offspring, which is useful to the diversity, species biology and natural history study (Felsenstein, 1985; Harvey & Pagel, 1991). We used the outgroup pruned tree to obtain the phylogenetic relationship variance-covariance matrix. With the phylogenetic tree, the variance-covariance matrix, and the morphological and behavioural data obtained for each Odonata species, we perform a series of phylogenetic comparative analyses to find the relationship between the adult sex ratio variation and natural and sexual selective forces.

Figure 1. Posterior probability consensus and ultrametric topology of 99 Odonata species, obtained by a posterior probability analysis of molecular evidence (see methods). On each internal node indicate their posterior probability. The node of Anisoptera and Zygoptera suborder are indicated. In the tips are the species name and their code of the adult sex ratio. The “A” columns refers to the adult sex ratio at emergence and the “B” column refers to the adult sex ratio at sexual maturity. In the “A” and “B” columns, blue indicate adult sex ratio biased to males; grey adult sex ratio whit out sex bias, and red, adult sex ratio biased to females.

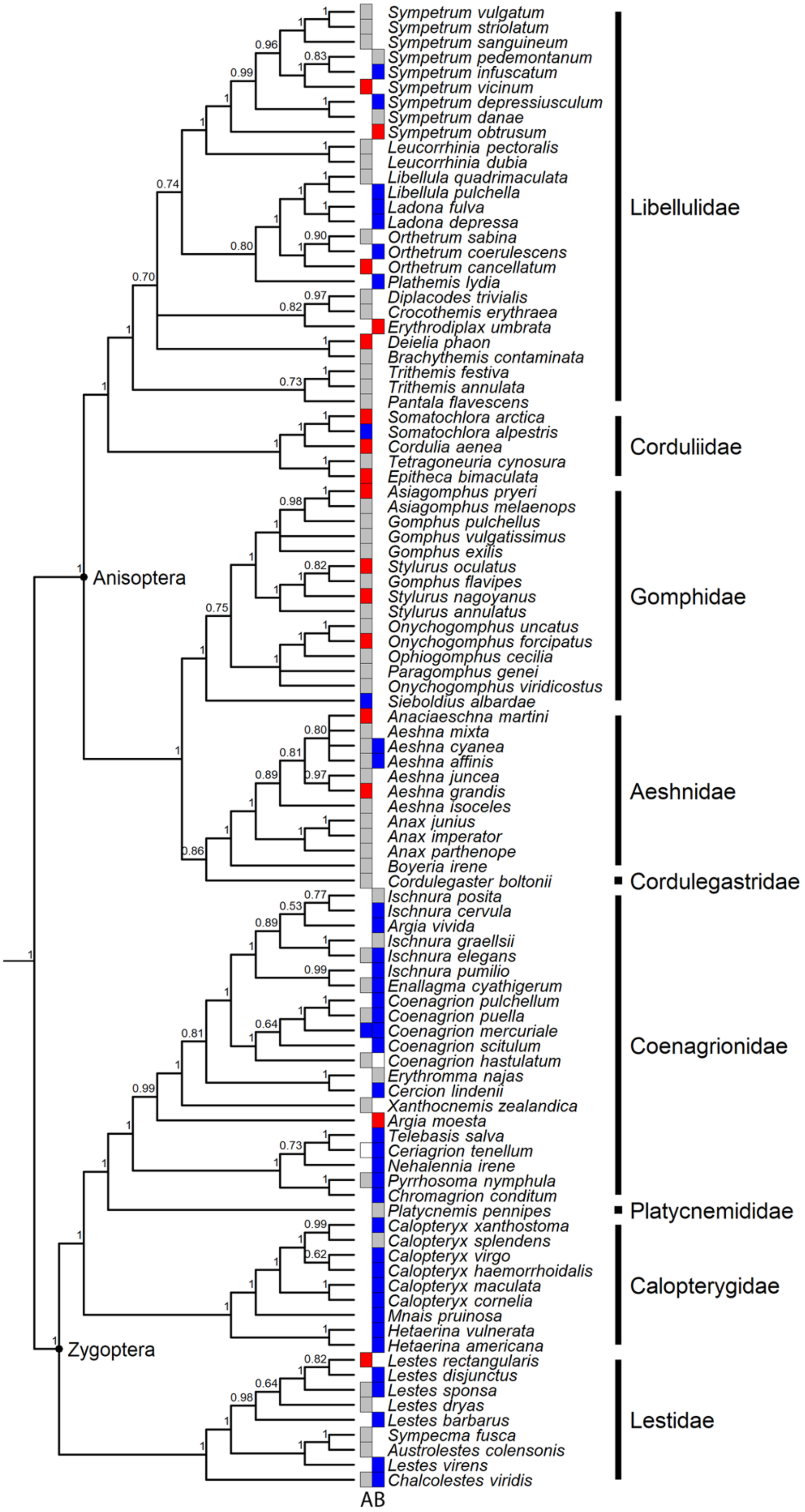
Sympetrum vulgatum
Sympetrum striolatum
Sympetrum sanguineum
Sympetrum pedemontanum
Sympetrum infuscatum
Sympetrum vicinum
Sympetrum depressiusculum
Sympetrum danae
Sympetrum obtrusum
Leucorrhinia pectoralis
Leucorrhinia dubia
Libellula quadrimaculata
Libellula pulchella
Ladona fulva
Ladona depressa
Orthetrum sabina
Orthetrum coerulescens
Orthetrum cancellatum
Plathemis lydia
Diplacodes trivialis
Crocothemis erythraea
Erythrodiplax umbrata
Deielia phaon
Brachythemis contaminata
Trithemis festiva
Trithemis annulata
Pantala flavescens
Somatochlora arctica
Somatochlora alpestris
Cordulia aenea
Tetragoneuria cynosura
Epitheca bimaculata
Asiagomphus pryeri
Asiagomphus melaenops
Gomphus pulchellus
Gomphus vulgatissimus
Gomphus exilis
Stylurus oculatus
Gomphus flavipes
Stylurus nagoyanus
Stylurus annulatus
Onychogomphus uncatus
Onychogomphus forcipatus
Ophiogomphus cecilia
Paragomphus genei
Onychogomphus viridicostus
Sieboldius albardae
Anaciaeschna martini
Aeshna mixta
Aeshna cyanea
Aeshna affinis
Aeshna juncea
Aeshna grandis
Aeshna isoceles
Anax junius
Anax imperator
Anax parthenope
Boyeria irene
Cordulegaster boltonii
Ischnura posita
Ischnura cervula
Argia vivida
Ischnura graellsii
Ischnura elegans
Ischnura pumilio
Enallagma cyathigerum
Coenagrion pulchellum
Coenagrion puella
Coenagrion mercuriale
Coenagrion scitulum
Coenagrion hastulatum
Erythromma najas
Cercion lindenii
Xanthocnemis zealandica
Argia moesta
Telebasis salva
Ceriagrion tenellum
Nehalennia irene
Pyrrhosoma nymphula
Chromagrion conditum
Platycnemis pennipes
Calopteryx xanthostoma
Calopteryx splendens
Calopteryx virgo
Calopteryx haemorrhoidalis
Calopteryx maculata
Calopteryx cornelia
Mnais pruinosa
Hetaerina vulnerata
Hetaerina americana
Lestes rectangularis
Lestes disjunctus
Lestes sponsa
Lestes dryas
Lestes barbarus
Sympecma fusca
Austrolestes colensonis
Lestes virens
Chalcolestes viridis
Libellulidae
Corduliidae
Gomphidae
Aeshnidae
Cordulegastridae
Coenagrionidae
Platycnemididae
Calopterygidae
Lestidae
Anisoptera
Zygoptera
AB

Figure 1.

Correlation between ASR and population sample size

First to start the phylogenetic comparative analysis, we made a correlational analysis between ASR and sample size (average of a number of individuals for each population of each specie), measured as the log_{10} transformed values for each specie, to examine if the population size influences the estimates of ASR. If both variable, ASR and sample size, not correlate, means that the ASR estimates are independent of the sample size. Also, we fitted a Phylogenetic Generalized Least Square (PGLS) analysis to correct by phylogeny if this association are evolutionary influenced (Supplementary Material 3, Table S1, S2 and S3) with "caper" package (Orme, 2018) implemented in R software (R Core Team, 2019). The PGLS method incorporates the phylogenetic autocorrelation of the species data in the structure of error (Martins & Hansen, 1997; Freckleton et al., 2002) and use the phylogenetic history of taxa to specify the structure of the variance-covariance matrix (see Rohlf, 2001). To improve the fit of our data to the model, we estimated the λ parameter by maximum likelihood (Pagel, 1994, 1999). This analysis was made for all Odonata species together, and for both sets of Odonata species, the species with ASR estimated by exuviae picking and the species whit ASR estimated by mark-recapture method.

Adult sex ratio differences between families

To examine the significance of variation in the adult sex ratio at the level of families, one sample phylogenetic t-test for each Odonate set, by exuviae picking and by mark-recapture, was performed using "phytools" package (Revell, 2012) implemented in R software (R Core Team, 2019). A μ of 0.5 was set as the threshold for significant biases in the adult sex ratio (Fig. 2 A and B; Supplementary Martial 3, Table S4 and S5).

Models to test the variable sources linked to the adult sex ratio

To test if the behavioural and morphological variables taking account influence in an evolutionary way the ASR variation, we fitted Phylogenetic Generalized Least Squares models

(PGLS) with “caper” package (Orme, 2018) implemented in R software (R Core Team, 2019). The model inferences and the AIC values were obtained using the “MuMIn” package in R (Barton, 2019). We did the modelling for the both sets of Odonata species, the species with ASR estimated by exuviae picking and the species whit ASR estimated by mark-recapture method.

For each set of Odonata species, we perform a multi-predictor, saturated model to investigate the relationship between ASR as dependent variable, and MS, WP, BS and SSD as explanatory variables. We include all interaction between the factor and explanatory variables and then, we generate a series of models whit all the possible combinations between the explanatory variables, from the saturated model to the null model, in order to choose the best fit model by AICc (Supplementary Material 3, Table S6, S7, S8 and S9). We compared PGLS models using Akaike´s Information Criterion correcting for small sample size (AICc) (Burnham & Anderson, 2002, 2011; Symonds & Moussalli, 2011). This approach allows comparisons of competing models with lower values of AIC representing “better models”. The AIC penalizes the increases in the number of parameters in the model. We calculate the relative support for models by examining the Δ AICc score (the difference in AICc score between the best approximating model and each other candidate model). The relative strength of each putative model is ascertained by calculating its Akaike weight (wi) which can be considered analogous to the probability that that model is the best approximated model. The R2 was calculated based on the likelihood radio test. We compiled a list of the top model (those models whit Δ AICc score < 2). The influential effects were those where the 95% confidence interval around the parameter estimate did not cross zero (Burnham & Anderson, 2002).

For both set of Odonata species, interactions between MS and morphological traits were found. For the Odonata species with ASR estimates by exuviae picking, the best fitted model showed the MS in interaction with BS and MS in interaction with WP. In the best fitted model for the species with ASR estimates by mark-recapture method, the MS were in interaction with

SSD. As the MS was in interaction whit the morphological traits (see Results), we modelled each interaction apart to obtain the slopes of the territorial and non-territorials species.

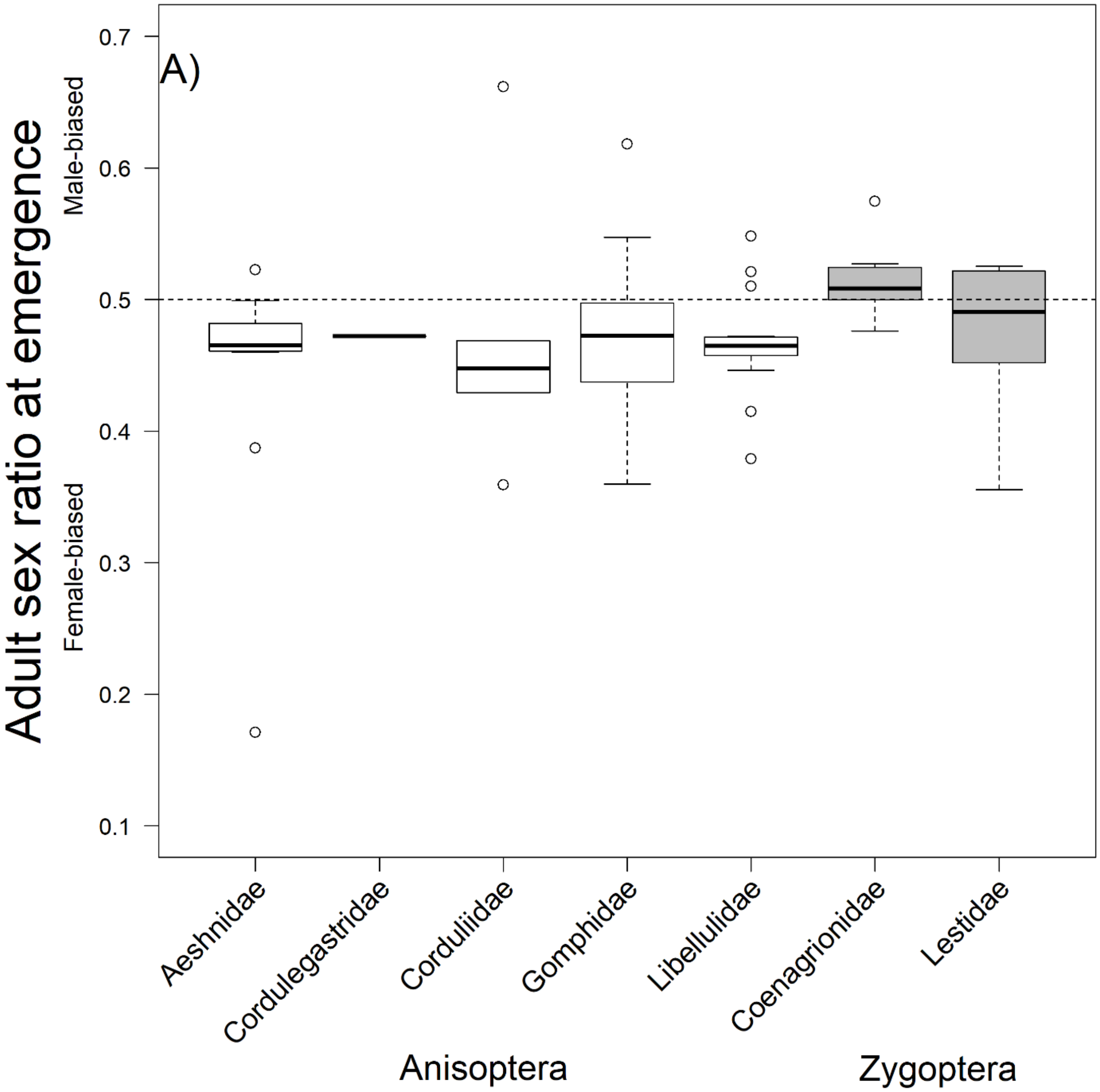

Figure 2 A.

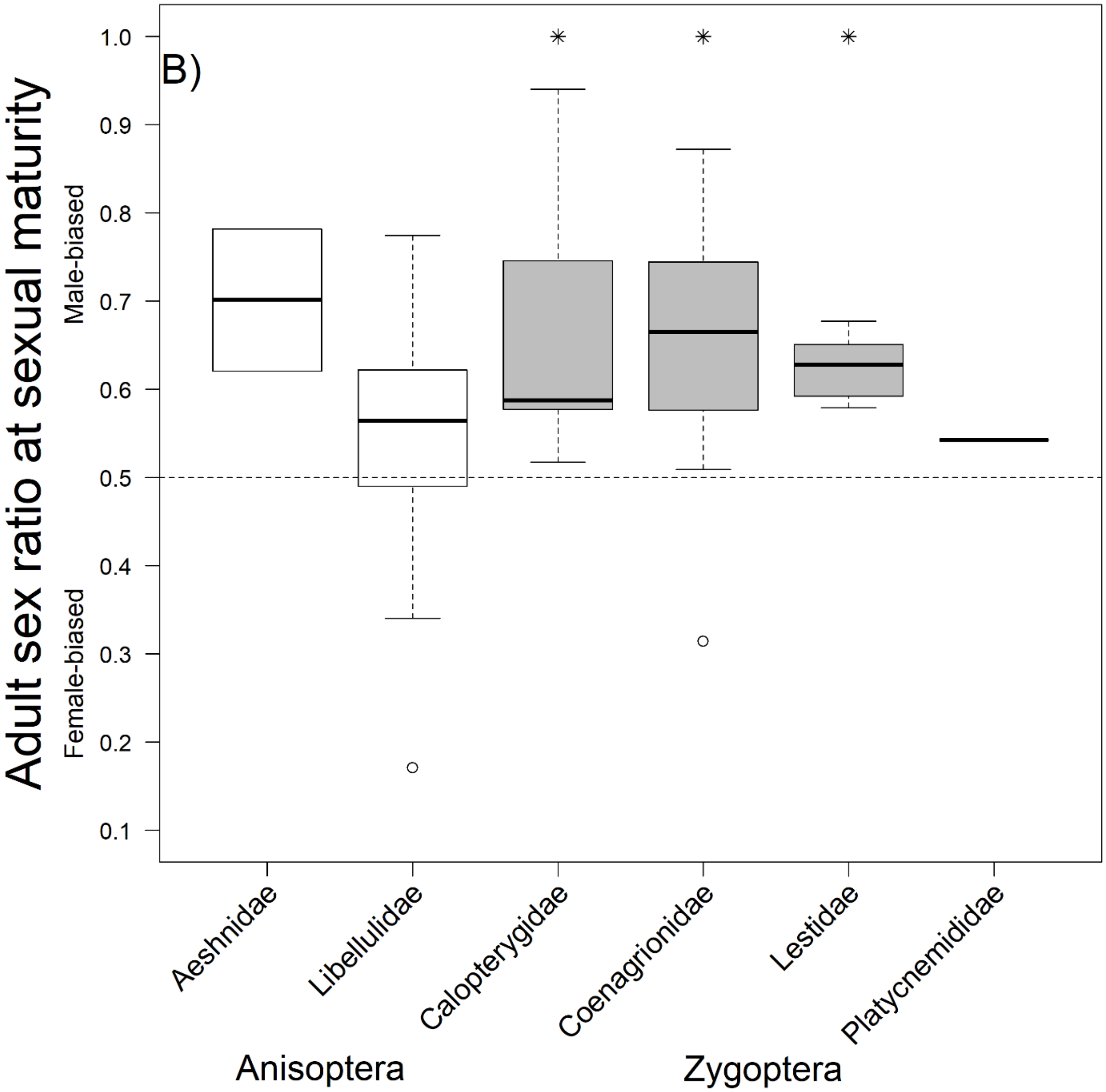

Figure 2 B.

Figure 2. Adult sex ratio between Odonata families (median, upper and lower quartiles; open circles are extreme values). The horizontal dotted line indicates ASR non-bias to any sex. The white boxes correspond to families of Anisoptera suborder and the grey boxes to families of Zygoptera suborder. A) ASR at emergence (exuviae picking method); B) AST at sexual maturity (mark-recapture method), the asterisk above Calopterygidae, Coenagrionidae and Lestidae families indicate that they ASR mean are different to non-bias (see Supplementary Material 3).

Results

Differences between methods

Both methods estimated the ASR differently (181 data of ASR of 62 species with ASR estimated by exuviae picking, and 92 data from 47 species whit ASR estimated by mark-recapture method (estimate (± S.E.): intercept = -0.97 (0.06), d.f. = 162, t = 17.26, P < 0.001; method = 0.21 (0.04), d.f. = 10, t = 5.80, P < 0.001). No relations were founded between methods for the 10 species with estimates of ARS by both methods (estimate (± S.E.), intercept = 0.47 (0.08), t = 6.1, P = 0.001; ASR by mark-recapture = 0.05 (0.11), t = 0.45, P = 0.67; Res.S.E.8 = 0.033; R2 = 0.024; F1,8 = 0.197; P = 0.67; slope test not equal to one, P = 0.67). The mean of the differences of both ASR methods were different (-0.18, 95% C.I. = -0.25 to -0.11, d.f. = 9, t =5.85, P <0.001).

Correlation between ASR and population sample size

In no case, all Odonata species, species with ASR estimated by exuviae picking or by mark-recapture method, the ASR estimates were not correlated with the species sample size (All Odonata species: estimate = -0.017, t = 0.166, d.f. = 97, P = 0.869; Exuviae picking: estimate = 0.232, t = 1.848, d.f. = 60, P = 0.69; Mark-recapture: estimate = 0.151, t = 0101, d.f. = 45, P = 0.920). We could not see correlation either when we integrate the phylogenetic information (Supplementary Material 3, Table S1, S2 and S3).

Differences between families

The distribution of the adult sex ratios in Odonata was widely distributed, from male to female bias (between families-phylogenetic t-test and PGLS). Any family showed a bias to male or to female in the set of Odonata species with ASR estimated by exuviae picking (Fig. 2 A, and Supplementary Material 3, Table S4), in contrast, at sexual maturity, three zygopteran families showed an evident male bias (Calopterygidae: 66%, Coenagrionidae 66%, and Lestidae: 63% of males, with lambda of 0.58, zero and zero, respectively), while an Anisoptera families showed a non-significant light bias to males (Libellullidae). The remaining families, one of Anisoptera (Aeshnidae) and one Zygoptera (Platycnemididae) could not be tested (Fig. 2 B and Supplementary Material 3, Table S5).

Behavioural and morphological indicators of sexual selection

Territorial MS was more common among Anisoptera species than in Zygoptera, nevertheless, one Zygoptera family was entirely territorial (see Supplementary Material 1). WP was present in species of both suborders. Of the 59 Anisoptera species, there were 30 with WP. In Zygoptera only 9 of the 40 species had pigment, but in this suborder the percentage was higher in one family (see Supplementary Material 1). Anisoptera species had an overall larger BS with lower SSD, meanwhile Zygoptera species were generally of small BS and some families had strong SSD (see Supplementary Material 1).

In the best PGLS fitted model to explain the variation of ASR in the Odonata species with ASR obtained from exuviae picking, the BS and WP correlates whit ASR variation, differently between the territorial and non-territorial species, while the SSD was not related (Table 1 and Supplementary Material 3, Table S6 and S7). Both morphological traits, the BS and WP, in the non-territorial and territorial species were negatively correlated with the evolutionary changes in ASR, from female bias to male bias, but the effect was greater in the former than the later ones, and none of these models there was a phylogenetically signal (Table 2, 3, 4 and 5). In the other hand, in the best PGLS fitted model to explain the variation of ASR in the Odonata species with ASR obtained from mark-recapture method, only the SSD correlates whit ASR variation, differently between the territorial and non-territorial species, while the BS or WP were not related (Table 6 and Supplementary Material 3, Table S8 and S9). The direction of the evolutionary effects of SSD over ASR variation in non-territorial species are negative, this is the only model that showed phylogenetic signal. Meanwhile, in territorial species the effects at evolutionary level are positive, and showed no phylogenetic signal (Table 7 and 8).

Table 1. Parameter estimates from best fitted model (AICc value and lowest number of parameters) explaining ASR variation among Odonata recently emerged as adults (exuviae method; N=48 species, λ=0).

	Estimate ± S.E.	*t*	95% C.I.
Intercept	1.856 ± 0.219	8.458	**1.427 - 2.286**
Mating System	-1.387 ± 0.249	5.712	**-1.875 - -0.899**
Body Size	-0.887 ± 0.138	6.432	**-1.157 - -0.617**
Sexual Size Dimorphism	0.327 ± 0.325	0.931	-0.310 – 0.964
Male Wing Pigment Proportion	7.110 ± 2.157	3.297	**2.882 - 11.337**
Mating System * Body Size	0.896 ± 0.151	5.949	**0.600 - 1.192**
Mating System * M. Wing Pigment P.	-7.195 ± 2.157	3.336	**-11.423 - -2.967**

Influential parameters (i.e. whose confidence intervals do not include zero) are shown in bold.

Table 2. PGLS model of the non-territorial Odonata recently emerged as adults and BS (exuviae method; N=15 species, λ=0).

	Estimate ± S.E.	*t*	95% C.I.
Intercept	1.383 ± 0.238	5.814	**0.917 – 1.849**
Body Size	-0.582 ± 0.147	3.972	**-0.870 - -0.294**

Influential parameters (i.e. whose confidence intervals do not include zero) are shown in bold.

Table 3. PGLS model of the territorial Odonata recently emerged as adults and BS (exuviae method; N=33 species, λ=0).

	Estimate ± S.E.	*t*	95% C.I.
Intercept	0.487 ± 0.101	4.811	**0.289 – 0.685**
Body Size	-0.004 ± 0.059	0.061	-0.112 – 0.112

Influential parameters (i.e. whose confidence intervals do not include zero) are shown in bold.

Table 4. PGLS model of the non-territorial Odonata recently emerged as adults and WP (exuviae method; N=15 species, λ=0).

	Estimate ± S.E.	*t*	95% C.I.
Intercept	0.447 ± 0.027	16.564	**0.394 - 0.500**
Male Wing Pigment Proportion	-2.053 ± 3.363	0.611	-8.644 - -4.538

Influential parameters (i.e. whose confidence intervals do not include zero) are shown in bold.

Table 5. PGLS model of the territorial Odonata recently emerged as adults and WP (exuviae method; N=33 species, λ=0).

	Estimate ± S.E.	*t*	95% C.I.
Intercept	0.486 ± 0.007	65.130	**0.472 – 0.500**
Male Wing Pigment Proportion	-0.082 ± 0.036	2.277	**-0.152 - -0.011**

Influential parameters (i.e. whose confidence intervals do not include zero) are shown in bold.

Table 6. Parameter estimates from best fitted model (AICc value and lowest number of parameters) explaining ASR variation among Odonata sexually mature (mark-recapture method; N=45 species, λ=0).

	Estimate ± S.E.	*t*	95% C.I.
Intercept	0.734 ± 0.388	1.891	-0.026 - 1.494
Mating System	0.016 ± 0.056	0.280	-0.094 - 0.126
Body Size	-0.067 ± 0.257	0.262	-0.571 - 0.437
Sexual Size Dimorphism	-1.274 ± 1.033	1.233	-3.299 - 0.751
Male Wing Pigment Proportion	-0.087 ± 0.071	1.230	-0.226 - 0.052
Mating System*Sexual Size Dimorphism	2.840 ± 1.404	2.023	**0.088 - 5.592**

Influential parameters (i.e. whose confidence intervals do not include zero) are shown in bold.

Table 7. PGLS model of ASR of the non-territorial Odonata sexually matured and SSD (mark-recapture method; N=22 species, λ=0.502).

	Estimate ± S.E.	*t*	95% C.I.
Intercept	0.596 ± 0.059	10.058	**0.480 – 0.712**
Sexual Size Dimorphism	-0.479 ± 0.940	0.510	-2.321 – 1.363

Influential parameters (i.e. whose confidence intervals do not include zero) are shown in bold.

Table 8. PGLS model of ASR of the territorial Odonata sexually matured and SSD (mark-recapture method; N=23 species, λ=0).

	Estimate ± S.E.	*t*	95% C.I.
Intercept	0.614 ± 0.032	19.327	**0.551 – 0.677**
Sexual Size Dimorphism	1.382± 0.933	1.481	-0.564 – 3.328

Influential parameters (i.e. whose confidence intervals do not include zero) are shown in bold.

Discussion

The amounts of adult males and females, in Odonata has attracted the attention of life scientist since the end of the nineteenth century (e.g. Tümpell, 1899), during the twentieth century (e.g. Tyllard, 1905, 1917; Corbet, 1962; Lawton 1972), and to this day (e.g. Stoks, 2001b; Cordero-Rivera & Stoks, 2008). Even if some authors have proposed that these quantities may be an artefact due to differences in sexual behaviour between (e.g. Parr & Palmer, 1971; Garrison & Hafernick, 1981; Hinnekint, 1987), Corbet & Hoess (1988) and Stoks (2001a) have shown that the exuviae picking method and the mark-recapture method, respectively, estimate in a good way the adult size ratio (ASR). We tested ASR estimates by these two methods against their population sample size, and since they were not related, neither if we incorporate the phylogenetic information, we can conclude that the ASR estimates were taken account for this research were well assessed and will be similar to other populations of each species. The sexual ratios estimated at different life cycle stages can reflect information for these particular stages, that can be related or not to the next stage (Székely et al., 2014). The ASR by itself, is a demographic property of populations that arises from sex differences in mortality, maturation rates and movement patterns (Le-Galliard et al., 2005; Veran & Beissinger, 2009). Instead, the operational sex ratio (OSR) is given by individual adults' decisions whether to join the breeding population, and how they can remain in suitable conditions to participate in the reproduction (Clutton-Brock & Parker, 1992; Kokko & Jennions, 2008). We discuss the demeanour of ASR by picking exuviae and by mark-recapture methods and the possible causes of their evolutionary trends.

The major variation between the Odonata ASR founded by us, was due the time to estimate it, and depend on the method used to calculate the proportions between male and female organisms. The exuviae picking method reflects the ASR for newly adults (just emerged from the youth stage or tenerals) which are considered sexually immature and they are not part of the reproductive population for a while (Corbet & Hoess, 1998; Corbet, 1999), this method reveals almost non-bias in their ASR (one male for one female). In change, the mark-recapture method reflects the ASR only for sexually matured individuals (since the tenerals are excluded because they are very fragile to mark; Cordero-Rivera & Stoks, 2008), it shows that many species had a male bias in their ASR (more males than females in the populations). This pattern found, keep consistent if we incorporate phylogenetic-family information, non-bias for newly emergent adults and male-bias for the sexually matured individuals. The ASR evolutionary trend in dragonflies and damselflies are related with their mating system. For the newly emerged adults, the body size and the male wing pigmentation are evolutionary related different between territorial and non-territorial species. For the sexually matured individuals, is the body size differences between sexes (SSD) are the one that interacts differently between species with distinct mating system.

The Odonata insects are sexually diploids and genetic sex-determined organism (Kiauta, 1969), as expected an equal primary sex ratio (Fisher, 1930; Hamilton, 1967). The larva stage specializes in growth (Wilbur, 1980) and face several stressors simultaneously (Shin et al., 2004; Stoks et al., 2008), but the bias in the sex ratios in the early stages not necessarily translate into bias in adulthood, and there are no predictions that say that their numbers must be matched (Székely et al., 2014). The ASR estimated by exuviae picking relate to selective forces and restrictions in early life cycle stages (e.g. predation risk in the larvae stage, Price, 1988; or time constraints to emerge, Johansson & Rowe, 1999) and allows measure sex ratios before any bias by dispersal or differential adult mortality appears (Michiels & Dhondt, 1989; Corbet & Hoess, 1998). Deviation in ASR can start ecological and evolutionary processes that can feedback this same bias and the responses may involve settings that can only see on evolutionary scales (Székely et al., 2014). This feedback can be positive or negative (Lehtonen & Kokko, 2012), a bias in the ASR may increase mortality in the more common sex and thus the bias may be self-corrected (Fisher, 1930; Hamilton, 1967) or reinforcement, for example, if the males develop secondary sexual traits that make them more attractive for females, but also more susceptible to depredation and starvation, a bias to females in their ASR will remain (Jennions & Kokko, 2010). Even if some populations present sexual differences in maturation rates or development times (protoandry in some populations: males emerge before females, e.g. De-Block & Stoks 2003; protogynadry in others populations: females emerge before males, e.g. Jodicke, 1997), generally, both sexes emerge in a synchronized way (Corbet, 1999). The ASR variation at emergence between species were wide between species (17-66% of males), similar to what has already been reported for several authors (e.g. slightly biased to males in Zygoptera and female biased in Anisoptera, Lawton, 1972; Corbet y Hoess, 1998), but when we incorporated the phylogenetic information a non-biased ASR patterns remain for all the families represented, similar to what was found by Jodicke (1997). Have a sex ratio without bias at the adult stage can be advantageous for both sexes, as each individual will have the chance to reproduce (Hamilton, 1967).

The evolutionary change in the ASR bias at emergence (exuviae picking) related to the body size was different between territorial and non-territorial species. The species tend to change from female bias to male bias in their ASR as its size decreases, but the effect is greater in non-territorial than in territorial ones. In many organisms, the variety in their sex ratios emerges until in the adulthood (Székely et al., 2014) and should influence the mating systems and mate acquisitions (Kokko & Jennions, 2008). In organism with complex life cycle organism, their adult body size is reached by the larval growth rate and the development time in each larval phase (Blanckenhorn, 2005) and present a trade-off between reaching maturity at an early age and achieve a large size, that under environmental-time constraints the mass at emergence can be affected, but the body size keeps constant (Abrams et al., 1996; Strobbe & Stoks, 2004), considerate as an adaptive phenotypic plasticity (Gotthard & Nylin, 1995). The size in Odonata is fixed a few hours after emergence (Corbet, 1999), which limits weight gain and flight performance, so that has to be optimized during larval stages (Strobbe & Stoks, 2004), and it can be related to the adult fitness (Banks & Thompson, 1987; Moore, 1990; Anholt, 1991; Cordero-Rivera, 1991; Crowley & Johansson, 2002). In Odonata there are a general fitness benefits to large size in adulthood, live longer, have greater mating rates and fecundity, also it would provide more space for fat to be built and stored; although there are some other detriments, as the predation and starvation risks (Zahavi, 1975; Schmidt-Nielsen, 1984;

Andersson, 1994; Plaistow & Siva-Jothy, 1999; Sokolovska et al., 2000; Crowley & Johansson, 2002; Thompson & Fincke, 2002), but evidence of non-size effect (e.g. Marden, 1989) and benefits to be small also exist (e.g. Convey, 1989), while these benefits are probably more important in scrambling species, because permit allocates more energetic resources to searching for potential mates, they may also operate in territorial species who need greater manoeuvrability to elaborate displays to woo their potential partners (Fairbairn & Preziosi, 1994; Blanckenhorn et al., 1995; De-Block & Stoks, 2007). For females, being bigger are beneficial for fecundity until a certain point where it is no longer advantageous (e.g. Thompson 1989 & Cordero-Rivera, 1995; Honek, 1993). For territorial males, being bigger can allow them to win contest for access to couples when the ASR ir female biased (Moore, 1990; Fairbairn & Preziosi, 1994; Sokolovska et at., 2000), but if the density of males rises over the females, the territorial defense no longer pays the cost, and the relation of body size and mating success disappear (Koenig, 1990; Fincke et al., 1997; Corbet, 1999; Cordero-Rivera, 1999; Kokko & Rankin, 2006; Raihani et al., 2008b; Suhonen et al., 2008). If the size no longer confers reproductions improvements for males, their development time can be adjusted to decrease the famine and depredation risk in early development stages, so the sexual proportions can bias to males (Crowley, 2000; Crowley & Johansson, 2002). In species who realize elaborate displays to attract females, generally, the males are small and need optimizes their body size in relation to their flight costs (Blanckenhorn et al., 1995), in this species, the females are the ones that choose their partner (Córdoba-Aguilar et al., 2015). In the no-territorial species, be larger also confers benefits in mating success and survival (Sokolovska et al., 2000), and this benefits seems to be greater to female (Anholt et al., 1991). If there are many females it is not necessary to look for them with such determination or move a lot to find them and the selection for fecundity in females can be greater than the necessity of be more effective in the couple search flight. But if there are few females, the males will have to search by them and the traits linked with searching and mobility would be selected by sexual selection and favor a small body size (Andersson, 1994; Blanckenhorn et al., 1995, Serrano-Meneses, 2008a, b) up to a certain limit (Stoks, 2000). Our results are in concordance with those of Johansson et al (2005) who found that the bias in the ASR are related whit the size of both sexes, joint with the Rench´s rule in this insect order (Abouheif & Faribairn, 1997; Serrano-Menses et al., 2008a). Nevertheless, more work is certainly needed to establish the relationship between size at emergence, fitness components and sex ratios (Fincke et al. 1997).

If happen an evolutionary augment in the male wig pigment proportion, the evolutionary trend in ASR is to change to female bias, more markedly in non-territorial species than in territorial ones. Another trait, as the secondary sexual traits are, which develops differently between sexes also can affect the ASR variation (Arnqvist & Rowe, 2005; Fairbairn et al., 2007; Székely et al., 2014). Some Odonata species develop a morphological trait, which it is known that behave as sexual trait in some species, a pigment in their wings, (Corbet, 1999; Serrano-Meneses, 2008c). This color trait is very variable between species, in form, pattern and color and is driven by sexual selection of male-male competition and by female choice (Siva-Jothy, 1999; Córdoba-Aguilar, 2002) and, generally, this wing pigmentation is more conspicuous in males than females (Corbet, 1999). It can be used as a specific or sexual identifier (Koskimaki et al., 2004; Rüppell et al., 2005; Córdoba-Aguilar & Cordero-Rivera, 2005; Contreras-Garduño et al., 2006; Svensson & Gosden, 2007). This trait generates advantages and risk for the carriers. In territorial species, the males in good condition may produce lager pigmented areas (Grether, 1997) which correlate with fat reserves, winning a territorial contest and mating success (Moore, 1990; Grether, 1996; 1997; Hooper et al., 1999; Siva-Jothy, 1999; Córdoba-Aguilar, 2002; Contreras-Garduño et al., 2006; Contreras-Garduño et al., 2007; Serrano-Meneses et al., 2007), in non-territorial species, it is not known very well, but they evolution are related to the intensity of sexual selection as in territorial species (Carrillo-Muñoz & Serrano-Meneses, unpublished). But also, the more ornamented individuals may be seen easily by their prey (Grether & Grey, 1996) and predators (MckPeek, 2008; Svensson & Friberg, 2007; Rantala et al., 2011) or have a trade off in their immune system (Forbes y Robb, 2008) (Siva-Jothy, 1999; Córdoba-Aguilar & Cordero-Rivera, 2005), and consequently their survival is reduced (Grether & Grey, 1996; Siva-Jothy & Plaistow, 1999). So have a bigger wing spot may arise the mating opportunities, but reduce the survival of the bearer and if the males face this selective pressure and die sooner than females the ASR will biased to females.

The sex ratio estimated by mark-recapture method are similar to the operational sex ratio (OSR: ratio of sexually active males to females, Kvarnemo & Ahnesjo, 1996; Shuster & Wade, 2003) and has played a central role in sexual selection, breeding and mating system (McNamara et al., 2000; Székely et al., 2000; Jennions & Kokko, 2010). Biased OSR may emerge from biased or unbiased ASR (Székely et al., 2014) and can be the cause and consequence of mating systems (Székely et al., 2000; Donald, 2002). Odonata presented a general male bias ASR for the sexually matured organism for all the families represented. To explain such deviations has been proposed hypothesis based on a biased sex ratio at birth, sex differential mortalities of young and adults, sex-differential maturation times, and sex-differential dispersal and migration patterns, however, it is recognized that these are not sufficient to explain the patterns observed in wild populations (Stoks, 2001a, b). The males, as the more abundant sex, become the predominant competitors for access to mates and will be under stronger sexual selection (Kvarnemo & Ahnesjo, 1996).

The sexual differences in body sizes and shapes, behavior and ornaments can generate differences in their energy requirements and mortality influencing the bias in the ASR (Arnqvist & Rowe, 2005; Fairbairn et al., 2007; Székely et al., 2010). We found in sexually matured Odonata, that the sexual size dimorphism (SSD) interacts differently between species with distinct mating system. The territorial species present an evolutionary change from female bias to male bias in their ASR when the evolutionary change is from female biased SSD to male biased SSD. Meanwhile, in the non-territorial species the evolutionary tendency is contrary. The patterns of SSD change according to mating system and male flight agility and are linked to sexual pressures (Serrano-Meneses et al., 2008a, b). The fecundity selection is the principal responsible when the females are bigger than the males (Andersson, 1994), and happen if the females have higher reproductive success because of their higher capacity for producing eggs (Honek, 1993), and/or if large females are preferred by males (Legaspi & Legaspi, 2005). In Odonata, generally the females are bigger than males, but this can be less evident or even opposite in territorial species (Anholt et al., 1991; Fincke et al., 1997). The sexual selection and the survival are the main responsible when the males are the biggest, as they, generally, can live longer, have greater mating rates (Zahavi, 1975; Schmidt-Nielsen, 1984; Andersson, 1994; Plaistow & Siva-Jothy, 1999; Sokolovska et al., 2000; Crowley & Johansson, 2002; Thompson & Fincke, 2002), and store more fat to fuel aerial fights (Marden & Waage, 1990; Plaistow & Siva-Jothy, 1996; Plaistow & Tsubaki, 2000; Contreras-Garduño et al., 2006). Also the sexual selection in males can modify the body size to be small if they make an elaborate display to attract mates or perform an active searching of mates (Andersson & Norberg, 1981; Fincke, 1988; Hakkarainen et al., 1996; Blomqvist et al., 1997; Raihani et al., 2008a; Serrano-Meneses & Székely, 2006). For territorial species can be advantageous be smaller than females if the cost of fight to mate is not payed, that is, when are sufficient females to each male, or if they perform an elaborate display who need agility, or defend their territories in flight and rarely perch, and can be able to allocate more time to sexual activity since small body size could have lower flying costs per unit time (Koenig & Albano, 1987; Van-Buskirk, 1987; Convey, 1989), but if the males outnumber females, the sexual selection via male-male contest increase and be bigger can be advantageous as in this species having a territory is often a prerequisite for males to obtain copulations, because females are attracted to these areas for copulation and/or oviposition (Corbet, 1999; Córdoba-Aguilar & Cordero Rivera, 2005; Suhonen et al., 2008). For non-territorial species, be smaller than females are probably because the fecundity selection is bigger than other selection in males and the sexual selection can select small male sizes if they are scramblers for females (Banks & Thompson, 1985; Anholt, 1991) because they may have lower flight costs per unit time than large males and therefore may allocate more time to active search for females instead of foraging (Blanckenhorn et al., 1995). Nevertheless, if males are less active and the female number is high, they no need search actively for finding a mate and no need be optimizing their size to be small, ant the benefits to be large remains (e.g. survival), in this case SSD without bias or male bias were the commonest (Stoks, 2000; e.g. *Hesperagrion heterodoxum*, Carrillo-Muñoz et al., unpublished). It should be noted that this model is the only who presented phylogenetic signal (λ=0.502), which is relatively high. For this subset, only three species are of Anisoptera suborder and the rest are Zygoptera species, the majority of Coenagrionidae species have a SSD biased to females and the rest, Platycnemididae and Lestidae species have SSD biased to males, if we were capable to model what happen in this species, maybe we can see a clear evolutionary patterns, but with our actual data set, the statistical power is reduced too much.

Two principal outcomes are shown with this research, firstly, the time to estimate the sex ratios is important, the sex ratio for newly emerged adults reflects no-bias to any sex, meanwhile the sex ratio estimated for the sexually matured adults show a general male bias. Both of this sex ratio can be related or not, but the information obtained from them are complementary. Second, both ASR is evolutionary influenced by the mating system and related whit the body size and wing permutation, and related to the sexual difference in body size, respectively. Nevertheless, with this outcome, we can´t know what happen between those two estimates, it makes necessary more effort to know the survival probabilities of adult of both sexes, as it can influence the bias in the ASR and the opportunities for sexual selection, and consequently the mating system used. Other sources of variation can affect the evolution of this sex ratio, as the genetic sex determination, the way of affront infections as *Wolbachia*, take into account if the populations are semelparous or iteroparous, and certainly rise the species to test, as the phylogenetic comparative methods are sensitive to the taxa and is possible that the slopes or patterns can change depending on the linages investigated.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

References

Abouheif, E. & Fairbairn D.J. (1997). A comparative analysis of allometry for sexual size dimorphism: assessing Rensch’s rule. The American Naturalist, 149, 540-562. http://doi.org/10.1086/286004

Abrams, P.A., Leimar, O., Nylin, S. & Wiklund, C. (1996). The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. The American Naturalist, 75, 381-395. http://doi.org/10.1086/282827

Altekar, G., Dwarkadas, S., Huelsenbeck, J.P. & Ronquist, F. (2004). Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics, 20, 407-415. https://doi.org/10.1093/bioinformatics/btg427

Andersson, M. & Norberg, R.A. (1981). Evolution of reversed sexual size dimorphism and role partitioning among predatory birds, with a size scaling of flight performance. Biological Journal of the Linnaean Society, 15, 105-130. http://doi.org/10.1111/j.1095-8312.1981.tb00752.x

Andersson, M. (1994). Sexual selection. New Jersey, USA: Princeton University Press.

Anholt, B.R. (1991). Measuring selection on a population of damselflies with a manipulated phenotype. Evolution, 45, 1091-1106. http://doi.org/10.1111/j.1558-5646.1991.tb04377.x

Arnqvist, G. & Rowe, L. (2005). Sexual conflict. New Jersey, USA: Princeton University Press.

Banks, M.J. & Thompson, D.J. (1985). Lifetime mating success in the damselfly Coenagrion puella. Animal Behaviour, 33, 1175-1183. http://doi.org/10.1016/S0003-3472(85)80178-0

Banks, M.J. & Thompson, D.J. (1987). Lifetime reproductive success of females of the damselfly Coenagrion puella. The Journal of Animal Ecology, 56, 815-832. http://doi.org/10.2307/4950

Barton, K. (2019). MuMIn: Multi-Model Inference. R package version 1.43.6. https://CRAN.R-project.org/package=MuMIn

Bessa-Gomes, C., Legendre, S. & Clobert, J. (2004). Allee effects, mating systems and the extinction risk in populations with two sexes. Ecology Letters, 7, 802-812. https://doi.org/10.1111/j.1461-0248.2004.00632.x

Blanckenhorn, W.U. (2005). Behavioural causes and consequences of sexual size dimorphism. Ethology, 111, 977-1016. http://doi.org/10.1111/j.1439-0310.2005.01147.x

Blanckenhorn, W.U. (2000). The evolution of body size: what keeps organisms small? The Quarterly Review of Biology, 75, 385-407. https://doi.org/10.1086/393620

Blanckenhorn, W.U., Preziosi, R.F. & Fairbairn, D.J. (1995). Time and energy constraints and the evolution of sexual size dimorphism - to eat or to mate? Evolutionary Ecology 9, 369-381. http://doi.org/10.1007/BF01237760

Blomqvist, D., Johansson, O.C., Unger, U., Larsson, M. & Flodin, L.A. (1997). Male aerial display and reversed sexual size dimorphism in the dunlin. Animal Behaviour, 54, 1291-1299. http://doi.org/10.1006/anbe.1997.0532

Burnham, K.P. & Anderson, D.R. (2002). Model selection and multimodel inference, 2nd ed. New York, USA: Springer.

Burnham, K.P. & Anderson, D.R. (2011). AIC model selection and multimodel inference in behavioural ecology: some background, observation, and comparison. Behavoural Ecology and Sociobiology. 65: 23-35. http://doi.org/10.1007/s00265-010-1029-6.

Bybee, S.M., Ogden, T.H., Branham, M.A. & Whiting, M.F. (2008). Molecules, morphology and fossils: a comprehensive approach to odonate phylogeny and the evolution of the odonate wing. Cladistics, 24, 477-514. https://doi.org/10.1111/j.1096-0031.2007.00191.x

Carle, F.L., Kjer, K.M. & May, M.L. (2008). Evolution of Odonata, with special reference to Coenagrionoidea (Zygoptera). Arthropod Systematics & Phylogeny, 66, 37-44

Carmona-Isunza, M.C., Ancona, S., Székely, T., Ramallo-González, A.P., Cruz-López, M., Serrano-Meneses, M.A. & Küpper, C. (2017). Adult sex ratio and operational sex ratio exhibit different temporal dynamics in the wild. Behavioral Ecology, 28, 523-532. https://doi.org/10.1093/beheco/arw183

Carrillo-Muñoz, A.I. & Serrano-Meneses, M.A (unpublished). Wing pigment evolution in dragonflies and damselflies (Insecta: Odonata).

Carrillo-Muñoz, A.I., García-Miranda, O., Córdoba-Aguilar, A., González-Soriano, E., Cueva-del-Castillo-Mendoza, R., Munguía-Steyer, R., García-Miranda, O. & Serrano-Meneses, M.A. (unpublished). Sexual size dimorphism in a non-territorial damselfly Hesperagrion heterodoxum.

Casale, P., Freggi, D., Basso, R. & Argano, R. (2005). Size at male maturity, se methods and adult sex ratio in loggerhead turtles (Caretta caretta) from Italian waters investigated through tail measurements. Herpetological Journal, 15, 145-148.

Clutton-Brock, T.H. & Parker, G.A. (1992). Potential reproductive rates and the operation of sexual selection. The Quarterly Review Biology, 67, 437-456. http://doi.org/10.1086/417793

Clutton-Brock, T.H. (1991). The evolution of parental care. New Jersey, USA: Princeton University Press.

Clutton-Brock, T.H. (2017). Reproductive competition and sexual selection. Philosophical Transactions of the Royal Society B: Biological Sciences, 372, 20160310. http://dx.doi.org/10.1098/rstb.2016.0310

Conrad, K.F. & Pritchard, G. (1992). An ecological classification of odonate mating systems: the relative influence of natural, inter- and intra-sexual selection on males. Biological Journal of the Linnean Society, 45, 255-269. https://doi.org/10.1111/j.1095-8312.1992.tb00643.x

Contreras-Garduño, J., Canales-Lazcano, J. & Córdoba-Aguilar, A. (2006). Wing pigmentation, immune ability, fat reserves and territorial status in males of the rubyspot damselfly Hetaerina americana. Journal of Ethology, 24, 165-173. http://doi.org/10.1007/s10164-005-0177-z

Contreras-Garduño, J., Lanz-Mendoza, H. & Córdoba-Aguilar, A. (2007). The expression of a sexually selected trait correlates with different immune defence components and survival in males of the American rubyspot. Journal of Insect Physiology, 53, 612-621. http://doi.org/10.1016/j.jinsphys.2007.03.003

Convey, P. (1989). Influences on the choice between territorial and satellite behavior in male Libellula quadrimaculata Linn (Odonata, Libellulidae). Behaviour, 109, 125-141. http://doi.org/10.1163/156853989X00187

Corbet, P.S. & Hoess, R. (1998). Sex ratio of Odonata at emergence. International Journal of Odonatology, 1, 99–118. http://doi.org/10.1080/13887890.1998.9748099

Corbet, P.S. (1999). Dragonflies: behaviour and ecology of Odonata. Essex, UK: Harley Books.

Corbet, P.S., (1962). A Biology of Dragonflies. London, UK: Witherby.

Cordero-Rivera, A., Lorenzo-Carballa, M.O., Utzeri, C. & Vieira, V. (2005). Parthenogenetic Ischnura hastata (Say), widespread in the Azores (Zygoptera: Coenagrionidae). Odonatologica, 34, 1-9.

Cordero-Rivera, A. (1991). Fecundity of Ischnura graellsii (Rambur) in the laboratory (Zygoptera: Coenagrionidae). Odonatologica, 20, 37-44.

Cordero-Rivera, A. (1995). Correlates of male mating success in two natural populations of the damselfly Ischnura graellsii (Odonata: Coenagrionidae). Ecological Entomology, 20, 213-222. http://doi.org/10.1111/j.1365-2311.1995.tb00450.x

Cordero-Rivera, A. (1999). Forced copulations and female contact guarding at a high male density in a Calopterygid damselfly. Journal of Insect Behavior, 12, 27–37. http://doi.org/10.1023/A:1020972913683

Cordero-Rivera, A. & Stoks, R. (2008). Mark-recapture studies and demography. In: Dragonflies and damselflies: model organisms for ecological and evolutionary research. (A. Córdoba-Aguilar, ed.), pp. 7-20. Oxford, UK: Oxford University Press.

Córdoba-Aguilar, A. (2002). Wing pigmentation in territorial male damselflies, Calopteryx haemorrhoidalis: a possible relation to sexual selection. Animal Behaviour, 63, 759-766. http://doi.org/10.1006/anbe.2001.1974

Córdoba-Aguilar, A. & Cordero-Rivera, A. (2005). Evolution and ecology of Calopterygidae (Zygoptera: Odonata): status of knowledge and research perspectives. Neotropical Entomology, 34, 861-879. http://dx.doi.org/10.1590/S1519-566X2005000600001

Córdoba-Aguilar, A., González-Tokman, D., Nava-Bolaños, A., Cuevas-Yañez, K., Rivas, M. & Nava-Sánchez, A. (2015). Female choice in damselflies and dragonflies. In: Cryptic Female Chioce in Arthropods, 1st Edition. (A. Peretti & A. Aisenberg, eds.), pp: 239-263, Switerland: Springer. http://doi.org/10.1007/978-3-319-17894-3_9

Crowley, P.H. (2000). Sexual dimorphism with female demographic dominance: age, size and sex ratio at maturation. Ecology, 81, 2592-2605. http://doi.org/10.1890/0012-6958(2000)081[2592:SDWFDD]2.0CO;2

Crowley, P.H. & Johansson, F. (2002) Sexual dimorphism in Odonata: age, size and sex ratio at emergence. Oikos, 96, 364-378. https://doi.org/10.1034/j.1600-0706.2002.960218.x

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. Nature Methods, 9, 772. https://doi.org/10.1038/nmeth.2109

Darwin, C. (1871). The descent of man, and selection in relation to sex. London, UK: John Murray.

De-Block, M. & Stoks, R. (2003). Adaptive sex-specific life history plasticity to temperature and photoperiod in a damselfly. Journal of Evolutionary Biology, 16, 986-995. http://doi.org/10.1046/j.1020-9101.2003.00581.x

De-Block, M. & Stoks, R. (2003). Flight-related body morphology shapes mating success in a damselfly. Animal Behaviour, 74, 1039-1098. http://doi.org/10.1016/j.anbehav.2007.01.0323

Dijkstra, K-D.B., Kalkman, V.J., Dow, R.A., Stokvis, F.R. & van-Tol, J. (2014). Redefining the damselfly families: a comprehensive molecular phylogeny of Zygoptera (Odonata). Systematic Entomology, 39, 68-96. https://doi.org/10.1111/syen.12035

Donald, P.F. (2007). Adult sex ratios in wild bird populations. Ibis, 149, 671-692. https://doi.org/10.1111/j.1474-919X.2007.00724.x

Dumont, H.J., Vierstraete, A. & Vanfleteren, J.R. (2010). A molecular phylogeny of the Odonata (Insecta). Systematic Entomology, 35, 6-18. https://doi.org/10.1111/j.1365-3113.2009.00489.x

Dyson, E.A. & Hurst, G.D.D. (2004). Persistence of an extreme sex-ratio bias in a natural population. Proceedings of the National Academy of Sciences of the United States of America, 101, 6520-6523. https://doi.org/10.1073/pnas.0304068101

Eberhart-Phillips, L.J., Küpper, C., Miller, T.E.X., Cruz-López, M., Maher, K.H., dos-Remedios, N., Stoffel, M.A., Hoffman, J.I., Krüger, O. & Székely, T. (2017). Sex-specific early survival drives adult sex ratio bias in snowy plovers and impacts mating system and population growth. Proceedings of the National Academy of Sciences of the United States of America, 114, E5474-E5481. https://doi.org/10.1073/pnas.1620043114

Fairbairn D.J. & Preziosi R.F. (1994). Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, Aquarius remigis. The American Naturalist, 144, 101-118. http://doi.org/10.1086/285663

Fairbairn, D.J., Blanckenhorn, W.U. & Székely, T. (2007). Sex, size and gender roles: evolutionary studies of sexual size dimorphism. Oxford, UK: Oxford University Press.

Felsenstein, J. 1985. Phylogenies and the comparative method. The American Naturalist, 125, 1-15. http://doi.org/10.1086/284325

Fincke, O.M. (1988). Sources of variation in lifetime reproductive success in a nonterritorial damselfly (Odonata: Coenagrionidae). In: Reproductive Success: Individual Variation in Constrasting Breeding Systems. (T.H. Clutton- Brock, ed.), pp. 22-43. USA, Chicago, USA: University of Chicago Press.

Fincke, O.M., Waage, J.K. & Koenig, W.D. (1997). Natural and sexual selection components of odonate mating patterns. In: The evolution of mating systems in insects and arachnids. (J.C. Choe & B.J. Crespi, eds.), pp. 58-74. Cambridge, UK: Cambridge University Press.

Fisher, R.A. (1930). The genetical theory of natural selection. Oxford, UK: Clarendon Press. http://doi.org/10.5962/bhl.title.27468

Forbes, M.R. & Robb, T. (2008). Testing hypotheses about parasite-mediated selection using odonate hosts. In: Dragonflies and damselflies: model organisms for ecological and evolutionary research (A. Córdoba-Aguilar, ed.), pp. 175-188. Oxford, UK: Oxford University Press.

Freckleton, R.P., Harvey, P.H. & Pagel, M. (2002). Phylogenetic analysis and comparative data: a test and review of evidence. The American Naturalist, 160, 712-726. http://doi.org/10.1086/343873

Garrison, R.W. & Hafernik, J.E. (1981). Population structure of the rare damselfly Ischnura gemina (Kennedy) (Odonata: Coenagrionidae). Oecologia, 48, 377-384. http://doi.org/10.1007/BF00346498

Godin, J.G.J. & McDonough, H.E. (2003). Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. Behavioral Ecology, 14, 194-200. https://doi.org/10.1093/beheco/14.2.194

Gotthard, K. & Nylin, S. (1995). Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. Oikos, 74, 3-17. http://doi.org/10.2307/3545669

Grether, G.F. (1996). Intrasexual competition alone favors a sexually dimorphic ornament in the rubyspot damselfly Hetaerina americana. Evolution, 50, 1949-1957. http://doi.org/10.1111/j.1558-5646.1996.tb03582.x

Grether, G.F. (1997). Survival cost of an intrasexually selected ornament in a damselfly. Proceedings of the Royal Society B: Biological Science, 264, 207-210. https://doi.org/10.1098/rspb.1997.0029

Grether, G.F. & Grey, R.M. (1996). Novel cost of a sexually selected trait in the rubyspot damselfly Hetaerina americana: conspicuousness to prey. Behavioral Ecology, 7, 465-473. https://doi.org/10.1093/beheco/7.4.465

Guindon, S. & Gascuel, O. (2003). A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology, 52, 696-704.

Hakkarainen, H., Huhta, E., Lahti, K., Lundvall, P., Mappes, T., Tolonen, P. & Wiehn, J. (1996). A test of male mating and hunting success in the kestrel: The advantages of smallness? Behavioral Ecology and Sociobiology, 39, 375-380. http://doi.org/10.1007/s002650050303

Hamilton, W.D. (1967). Extraordinary sex ratios. Science, 156, 477-488. http://doi.org/10.1126/science.156.3774.477

Harvey, P.H. & Pagel, M.D. (1991). The comparative method in evolutionary biology. Oxford, UK: Oxford University Press.

Hinnekint, B.O.N. (1987) Population dynamics of Ischnura e. elegans (Vander Linden) (Insecta: Odonata) with special reference to morphological colour changes, female polymorphism, multiannual cycles and their influence on behaviour. Hydrobiologia, 146, 3-31. http://doi.org/10.1007/BF00007574

Hirst, A.G., Bonnet, D., Conway, D.V.P. & Kiørboe, T. (2010). Does predation control adult sex ratios and longevities in pelagic copepods? Limnology and Oceanography, 55, 2193-2206. https://doi.org/10.4319/lo.2010.55.5.2193

Honek A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos, 66, 483-492. http://doi.org/10.2307/3544943

Hooper, R.E., Tsubaki, Y. & Siva-Jothy, M.T. (1999). Expression of a costly, plastic secondary sexual trait is correlated with age and condition in a damselfly with two male morphs. Physiological Entomology, 24, 364-369. http://doi.org/10.1046/j.1364/j.1365-3032.1999.00152.x

Huelsenbeck, J.P. & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics, 17, 754-755.

Jennions, M.D. & Kokko, H. (2010). Sexual selection. In: Evolutionary Behavioral Ecology (D. Westneat & C. Fox, eds.), pp. 343–364. Oxford University Press, New York.

Jodicke, R. (1997). Die Binsenjungfern und Winterlibellen Europas. Lestidae. Magdeburg Germany: Westarp Wiseenschaften.

Johansson, F. & Rowe, L. (1999), Life history and behavioral responses to time constraints in a damselfly. Ecology, 80, 1242-1252. http://doi.org/10.1890/0012-9658(2001)082[1857:LHPIAD]2.0.CO;2

Johansson, F., Crowley, P.H. & Brodin, T. (2005). Sexual size dimorphism and sex ratios in dragonflies (Odonata). Biological Journal of the Linnean Society, 86, 507-513. https://doi.org/10.1111/j.1095-8312.2005.00549.x

Kalmbach, E. & Benito, M.M. (2007). Sexual size dimorphism and offspring vulnerability in birds. In: Sex, size and gender roles (D.J. Fairbairn, W.U. Blanckenhorn & T. Székely, eds.), pp. 133-142. Oxford, UK: Oxford University Press.

Kiauta, B. (1969). Sex chromosomes and sex determination mechanisms in Odonata, with a review of the cytological conditions in the family Gomphidae, and references to the karyotypic evolution of the order. Genetica, 40, 127-157. http://doi.org/10.1007/BF01787346

Knaus, P. (1999). Untersuchungen zur Emergenz, zur Mobilität und zum Paarungssystem an einer Metapopulation von Somatochlora alpestris (Selys 1840) in den Zentralalpen (Anisoptera: Corduliidae). Diplomarbeit, Universität Zürich.

Koenig, W.D. & Albano, S.S. (1987). Lifetime reproductive success, selection, and the opportunity for selection in the white-tailed skimmer Plathemis lydia (Odonata: Libellulidae). Evolution, 41, 22-36. http://doi.org/10.1111/j.1558-5646.1987.tb05768.x

Koenig, W.D. (1990). Territory size and duration in the white-tailed skimmer Plathemis lydia (Odonata: Libellulidae). Journal of Animal Ecology, 59, 317-333. https://doi.org/10.2307/5175

Kokko, H. & Jennions, M.D. (2008). Parental investment, sexual selection and sex ratios. Journal of Evolutionary Biology, 21, 919-948. https://doi.org/10.1111/j.1420-9101.2008.01540.x

Kokko, H. & Rankin, D.J. (2006). Lonely hearts or sex in the city? Density-dependent effects in mating systems. Philosophical Transactions of the Royal Society B: Biological Sciences, 361, 319–334. http://doi.org/10.1098/rstb.2005.1784

Koskimaki, J., Rantala, M.J., Taskinen, J., Tynkkynen, K. & Suhonen, J. (2004). Immunocompetence and resource holding potential in the damselfly, Calopteryx virgo L. Behavioral Ecology, 15, 169-173. http://doi.org/10.1093/beheco/arg088

Kusano, T. & Inoue, M. (2011). Adult sex ratio of a population of the Japanese Salamander, Hynobius tokyoensis (Caudata: Hynobiidae). Current Herpetology, 30, 129-135. https://doi.org/10.3105/hsj.30.129

Kvarnemo, C. & Ahnesjo, I. (1996). The dynamics of operational sex ratios and competition for mates. Trends in Ecology and Evolution, 11, 404-408. http://doi.org/.10.1016/0169-5347(96)10056-2

Lawton, J.H., (1972). Sex ratios in Odonata larvae, with particular reference to the Zygoptera. Odonatologica, 1, 209-219.

Le-Galliard, J.F., Fitze, P.S., Ferrière, R. & Clobert, J. (2005). Sex ratio bias, male aggression, and population collapse in lizards. Proceedings of the National Academy of Sciences of the United States of America, 102, 18231-18236. https://doi.org/10.1073/pnas.0505172102

Legaspi, J.C. & Legaspi, B.C. (2005). Body weights and egg loads in field-collected Podisus maculiventris (Heteroptera: Pentatomidae). Florida Entomologist, 88, 38-42. http://doi.org/10.1653/0015-4040(2005)088[0038:BWAELI]2.0.CO;2

Lehtonen, J. & Kokko, H. (2012). Positive feedback and alternative stable states in inbreeding, cooperation, sex roles and other evolutionary processes. Philosophical Transaction of the Roya Society B: Biological Sciences, 367, 211-221. http://doi.org/10.1098/rstb.2011.0177

Letsch, H., Gottsberger, B. & Ware, J.L. (2016). Not going with the flow: a comprehensive time-calibrated phylogeny of dragonflies (Anisoptera: Odonata: Insecta) provides evidence for the role of lentic habitats on diversification. Molecular Ecology, 25, 1340-1353. https://doi.org/10.1111/mec.13562

Liker, A. & Székely, T. (2005). Mortality costs of sexual selection and parental care in natural populations of birds. Evolution, 59, 890-897. https://doi.org/10.1111/j.0014-3820.2005.tb01762.x

Lovich, J.E. & Gibbons, J.W. (1992). A review of techniques for quantifying sexual size dimorphism. Growth, Development & Aging, 56, 269-281.

Maddison, W.P. & Maddison, D.R. (2018). Mesquite: a modular system for evolutionary analysis. Version 3.51. http://www.mesquiteproject.org.

Marden, J.H. & Waage, J.K. (1990). Escalated damselfly territorial contests are energetic wars of attrition. Animal Behaviour, 9, 954-959. http://doi.org/10.1016/S0003-3472(05)80960-1

Marden, J.H. (1989). Bodybuilding dragonflies: costs and benefits of maximizing flight muscle. Physiological Zoology, 62, 505-521. http://doi.org/10.1086/physzool.62.2.300156182

Martins, E.P. & Hansen, T.F. (1997). Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. The American Naturalist, 149: 646-667. http://doi.org/10.1086/286013

McPeek, M.A. (2008). Ecological factors limiting the distributions and abundances of Odonata. In: Dragonflies and damselflies: model organisms for ecological and evolutionary research (A. Córdoba-Aguilar, ed.), pp 51-62. Oxford, UK: Oxford University Press.

Michiels, N.K. & Dhondt, A.A. (1989). Effects of emergence characteristics on longevity and maturation in the dragonfly Sympetrum danae (Anisoptera: Libellulidae). Hydrobiologia, 171, 149-158. http://doi.org/10.1007/BF00008175

Moller, A.P. & Szép, T. (2002). Survival rate of adult barn swallows Hirundo rustica in relation to sexual selection and reproduction. Ecology, 83, 2220-2228. http://doi.org/10.2307/3072053

Moore, A.J. (1990). The evolution of sexual dimorphism by sexual selection: the separate effects of intrasexual selection an-dintersexual selection. Evolution, 44, 315-331. http://doi.org/10.1111/j.1558-5646.1990.tb05201.x

Müller, J., Müller, K., Nienhuis, C. & Quandt, D. (2012). PhyDe - Phylogenetic Data Editor. Programme distributed by the author: http://www.phyde.de/.

NCBI Resource Coordinators. (2018). Database resources of the National Center for Biotechnology Information. Nucleic Acids Research, 46, D8-D13. doi: 10.1093/nar/gkx1095

Normark, B.B. (2014). Modes of reproduction. In: The evolution of insect mating systems (D.M. Shuker & L.W. Simmons, eds.), pp. 1-19. Oxford, UK: Oxford University Press.

Orme, D. (2018). The caper package: comparative analyses of phylogenetics and evolution in R. R package version 0.5.2. See: http://R-Forge.R-project.org/projects/caper.

Osmundson, D.B. (2006). Proximate causes of sexual size dimorphism in Colorado pikeminnow, a long-lived cyprinid. Journal of Fish Biology, 68, 1563-1588. https://doi.org/10.1111/j.0022-1112.2006.001043.x

Pagel, M. (1994). Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proceedings of the Royal Society B: Biological Science. 255: 37-45. http://doi.org/10.1098/rspb.1994.0006

Pagel, M. (1999). Inferring the historical patterns of biological evolution. Nature, 401, 887-884. http://doi.org/10.1038/44766

Paradis, E., Claude, J. & Strimmer, K. (2004). Ape: analyses of phylogenetics and evolution in R language. Bioinformatics, 20, 289-290. https://doi.org/10.1093/bioinformatics/btg412

Parr, M.J. & Palmer, M. (1971). The sex ratios, mating frequencies and mating expectancies of three coenagriids (Odonata: Zygoptera) in Northern England. Insect Systematic and Evolution, 2, 191-204. http://doi.org/1.1163/187631271X00202

Pen, I., Uller, T., Feldmeyer, B., Harts, A., While, G.M. & Wapstra, E. (2010). Climate-driven population divergence in sex-determining system. Nature, 468, 436-438. https://doi.org/10.1038/nature09512

Pierce, C.L. (1988). Predator avoidance, microhabitat shift, and risk sensitive foraging in larval dragonflies. Oecologia, 77, 81-90. http://doi.org/10.1007/BF00380929

Plaistow, S. J. & Siva-Jothy, M.T. (1996). Energetic constraints and male mate–securing tactics in the damselfly Calopteryx splendens xanthostoma (Charpentier). Proceedings of the Royal Society B: Biological Science, 263, 1233-1239. http://doi.org/10.1098/rspb.1996.0181

Plaistow, S. J. & Tsubaki, Y. (2000). A selective trade-off for territoriality and nonterritoriality in the polymorphic damselfly Mnais costalis. Proceedings of the Royal Society B: Biological Science, 267, 969-975. http://doi.org/10.1098/rspb.2000.1098

Plasitow, S. & Siva-Jothy, M.T. (1999). The ontogenetic switch between odonate life history stages: effects on fitness when time and food are limited. Animal Behaviour, 58, 659-667. http://doi.org/10.1006/anbe.19999.1171

Poulin, R. (1997a). Covariation of sexual size dimorphism and adult sex ratio in parasitic nematodes. Biological Journal of the Linnean Society, 62, 567-580. https://doi.org/10.1111/j.1095-8312.1997.tb00322.x

Poulin, R. (1997b). Population abundance and sex ratio in dioecious helminth parasites. Oecologia, 111, 375-380. https://doi.org/10.1007/s004420050248

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Raihani, G., Serrano-Meneses, M.A. & Córdoba-Aguilar, A. (2008a). Male mating tactics in the American rubyspot damselfl y: territoriality, nonterritoriality and switching behaviour. Animal Behaviour, https://doi.org/10.1016/j.anbehav.2007.11.002.

Raihani, G., Székely, T., Serrano-Meneses, M.A., Pitra, C. & Goriup, P. (2006b). The influence of sexual selection and male agility on sexual size dimorphism in bustards (Otididae). Animal Behaviou, 71, 833-838. http://doi.org/10.1016/j.anbehav.2005.06.013

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology, 67, 901-904. https://doi.org/10.1093/sysbio/syy032

Rantala, M.J., Honkavaara, J., Dunn, D.W. & Suhonen, J. (2011). Predation selects for increased immune function in male damselflies, Calopteryx splendens. Proceedings of the Royal Society B: Biological Science, 278, 1231-1238. http://doi.org/10.1098/rspb.2010.1680

Revell, L.J. (2012). Phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution. 3, 217-223. http://doi.org/10.111/j.2041-210X.2011.00169.x

Robert, C.E. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32, 1792-1797. https://doi.org/10.1093/nar/gkh340

Rohlf, F.J. (2001). Comparative methods for the analysis of continuous variables: geometric interpretations. Evolution 55, 2143-22160. http://doi.org/10.1111/j.0014-3820.2001.tb00731.x

Ronquist, F. & Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19, 1572-1574. https://doi.org/10.1093/bioinformatics/btg180

Rüppell, G., Hilfert-Rüppel, D., Rehfeldt, G. & Schüte, C. (2005). Die prachtlibellen europas. Hohenwarsleben, Germany: Westrap, Wissenschaften.

Schmidt-Nielsen, K. (1984). Scaling: Why is animal size so important? Cambridge, UK: Cambridge University Press.

Serrano-Meneses, M.A. & Székely, T. (2006). Sexual size dimorphism in seabirds: sexual selection, fecundity selection and differential niche-utilization. Oikos, 113, 385-394. http://doi.org/10.1111/j.0030-1299.2006.1426.x

Serrano-Meneses, M.A., Córdoba-Aguilar, A., Azpilicueta-Amorín, M., González-Soriano, E. & Székely, T. (2008a). Sexual selection, sexual size dimorphism and Rensch's rule in Odonata. Journal of Evolutionary Biology, 21, 1259-1273. https://doi.org/10.1111/j.1420-9101.2008.01567.x

Serrano-Meneses, M.A., Córdoba-Aguilar, A. & Székely, T. (2008b). Sexual size dimorphism: patterns and processes. In: Dragonflies and damselflies: model organisms for ecological and evolutionary research. (A. Córdoba-Aguilar, ed.), pp. 231-247. Oxford, UK: Oxford University Press.

Serrano-Meneses, M.A., Córdoba-Aguilar, A., Méndez, V., Layen, S.J. & Szekely T. (2007). Sexual size dimorphism in the American rubyspot: male body size predicts male competition and mating success. Animal Behaviour, 73, 987-997. http://doi.org/10.11016/j.anbehav.2006.08.012

Serrano-Meneses, M.A., Sánchez-Rojas, G. & Córdoba-Aguilar, A. (2008c). Sexual selection as possible underlying force in calopterygid wing pigmentation: comparative evidence with Hetaerna and Calopteryx (Zygoptera: Calopterygidae). Odonatologica, 37, 22-233.

Sherratt, T.N. & Beatty, C.D. (2005). Island of the clones. Nature, 435, 1039-1040. http://doi.org/10.1038/4351039a

Shuster, S.M. & Wade, M.J. (2003). Mating systems and strategies. New Jersey, USA: Princeton University Press.

Sih, A., Bell, A. & Kerby, J. (2004). Two stressors are far deadlier than one. Trends in Ecology and Evolution, 19, 274-276. http://doi.org/10.1016/j.tree.2004.02.010

Siva-Jothy, M.T. & Plaistow, S.J. (1999). A fitness cost of eugregarine parasitism in a damselfly. Ecological Entomology, 24, 465-470. http://doi.org/10.1046/j.1365-2311.1999.00222.x

Siva-Jothy, M.T. (1999). Male wing pigmentation may affect reproductive success via female choice in a calopterygid damselfly (Zygoptera). Behaviour, 136, 1365-1377. http://doi.org/10.1163/156853999500776

Siva-Jothy, M.T. (2000). A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. Proceedings of the Royal Society B: Biological Science, 267, 2523–2527. http://doi.org/10.1098/rspb.2000.1315

Sokolovska N, Rowe L, Johansson F. (2000). Fitness and body size in mature odonates. Ecological Entomology, 25, 239-248. http://doi.org/10.1046/j.1365-2311.2000.00251.x

Solberg, E.J., Loison, A., Ringsby, T.H., Sæther, B.E. & Heim, M. (2002). Biased adult sex ratio can affect fecundity in primiparous moose Alces alces. Wildlife Biology, 8, 117-128. https://doi.org/10.2981/wlb.2002.016

Stoks, R. (2000). Components of lifetime mating success and body size in males of a scrambling damselfly. Animal Behaviour, 2000, 59, 339–348. http://doi.org/10.1006/anbe.1999.1309

Stoks, R. (2001a). Male-biased sex ratios in mature damselfly populations: real or artefact? Ecological Entomology, 26, 181-187. http://doi.org/10.1046/j.1365-2311.2001.00301.x

Stoks, R. (2001b). What causes male-biased sex ratios in mature damselfly populations? Ecological Entomology, 26, 188-197. http://doi.org/10.1046/j.1365-2311.2001.00303.x

Stoks, R., Johansson, F. & De-Block, M. (2008). Life-history plasticity under time stress in damselfly larvae. In: Dragonflies and damselflies: model organisms for ecological and evolutionary research (A. Córdoba-Aguilar, ed.). pp.39-50, Oxford, UK: Oxford University Press.

Strobbe, F. & Stoks, R. (2004). Life history reaction norms to time constraints in a damselfly: differential effects on size and mass. Biological Journal of the Linnean Society, 83, 187-196. http://doi.org/10.1111/j.1095-8312.2004.00379.x

Suhonen, J., Rantala, M.J. & Honkavaara, J. (2008). Territoriality in odonates. In: Dragonflies and damselflies: model organisms for ecological and evolutionary research. (A. Córdoba-Aguilar, ed.), pp. 203-217. Oxford, UK: Oxford University Press.

Svensson, E.I. & Friberg, M. (2007). Selective predation on wing morphology in sympatric damselflies. The American Naturalist 170, 101-112. http://doi.org/10.1086/518181

Svensson, E.I. & Gosden, T.P. (2007). Contemporary evolution of secondary sexual traits in the wild. Functional Ecology 21, 422-433. http://doi.org/10.1111/j.1365-2435.2007.01265.x

Svensson, E.I. & Waller, J.T. (2013). Ecology and sexual selection: evolution of wing pigmentation in Calopterygid damselflies in relation to latitude, sexual dimorphism, and speciation. The American Naturalist, 182, E174-E195. https://doi.org/10.1086/673206

Symonds, M.R.E. & Moussalli, A. (2011). A brief guide to model section, multimodel inference and model averaging in behavioural ecology using Akaike´s information criterion. Behavoural Ecology and Sociobiology. 65:13-21. http://doi.org/10.1007/s00265-010-1037-6

Székely, T., Webb, J.N. & Cuthill, I.C. (2000). Mating patterns, sexual selection and parental care: an integrative approach. In: Vertebrate Mating Systems (M. Apollonio, M. Festa-Bianchet & D. Mainardi, eds.), pp. 194-223. World Science Press, London. http://doi.org/10.1142/9789812793584_0008

Székely, T., Weissing, F. J. & Komdeur, J. (2014). Adult sex ratio variation: implications for breeding system evolution. Journal of Evolutionary Biology, 27, 1500-1512. https://doi.org/10.1111/jeb.12415

Thompson, D.J. (1989). Sexual size dimorphism in Coenagrion puella (L.) (Zygoptera: Coenagrionidae). Advances in Odonatology, 4, 123-131.

Thompson, D.J. & Fincke, O.M. (2002). Body size and fitness in Odonata, stabilising selection and a meta-analysis too far? Ecological Entomology, 27, 378-384. http://doi.org/10.1046/j.1365-2311.2002.00419.x

Tillyard, R.J. (1917). The biology of dragonflies. Cambridge, UK: Cambridge University Press.

Tillyard, R.J., (1905). On the supposed numerical preponderance of the males in Odonata. Proceedings of the Linnean Society of New South Wales 30: 344-349. http://doi.org/10.5962/bhl.part.12908

Toïgo, C. & Gaillard, J.-M. (2003). Causes of sex-biased adult survival in ungulates: sexual size dimorphism, mating tactic or environment harshness? Oikos, 101, 376-384. https://doi.org/10.1034/j.1600-0706.2003.12073.x

Tsubaki, Y., Hooper, R.E. & Siva-Jothy, M.T. (1997). Differences in adult and reproductive lifespan in the two male forms of Mnais pruinosa costalis Selys (Odonata: Calopterygidae). Researches on Population Ecology, 39, 149-155. https://doi.org/10.1007/BF02765260

Tmpel, R. (1899). Über das scheinbar seltene Vorkommen der Weibchen mancher Libellenarten. Illustrierte Zeitschrift für Entomologie, 4, 227-228.

Van-Buskirk, J. (1987). Influence of size and date of emergence on male survival and mating success in a dragonfly, Sympetrum rubicundulum. American Midland Naturalist, 118, 169-176. http://doi.org/10.2307/2425640

Veran, S. & Beissinger, S.R. (2009). Demographic origins of skewed operational and adult sex ratios: perturbation analyses of two-sex models. Ecology Letters, 12, 129-143. https://doi.org/10.1111/j.1461-0248.2008.01268.x

West, S.A. (2009). Sex allocation. New Jersey, USA: Princeton University Press.

Wheeler, W.C., Whiting, M., Wheeler, Q.D. & Carpenter, J.M. (2001). The phylogeny of the extant hexapod orders. Cladistics, 17, 113-169. https://doi.org/10.1006/clad.2000.0147

Wilbur, E.E. (1980). Complex life cycles. Annual Review of Ecology and Systematics, 11, 67-93. http://doi.org/10.1146/annurev.es.11.110180.000435

Wilson, E.O. (1975). Sociobiology: the new synthesis. Boston, USA: Harvard University Press.

Zahavi, A. 1975. Mate selection - a selection for a handicap. Journal of Theoretical Biology, 53, 205-214. http://doi.org/10.1016/0022-5193(75)90111-3

Supplementary Material 1

Table S1. Data captured for the 99 Odonata species taken account in this study. ASRE: adult sex ratio at emergence; ASRM: adult sex ratio by mark-recapture; MTL: male total length in mm; FTL: female total length in mm.; WPP: male wing pigment proportion; MS: mating system, 0 = non-territorial, 1 = territorial.

Species	Suborder	ASRE	ASRM	MTL	FTL	WPP	MS	References
Aeshna affinis	Anisoptera	0.5	0.78	59.2	61	0	1	19, 110, 162, 194, 195, 198, 202
Aeshna cyanea	Anisoptera	0.48	0.62	73.4	72.5	0	1	22, 34, 67, 77, 110, 176, 179, 187, 194, 195, 217
Aeshna grandis	Anisoptera	0.39	---	71.7	68	1	1	75, 133, 154, 179, 195
Aeshna isoceles	Anisoptera	0.52	---	64.8	68.7	0.03	1	22, 27, 93, 133, 152, 179, 194, 195, 202, 206, 219, 223
Aeshna juncea	Anisoptera	0.46	---	72.8	68.5	0	1	110, 176, 194, 195, 203, 218, 221
Aeshna mixta	Anisoptera	0.46	---	63.9	60.9	0	1	22, 34, 73, 110, 176, 194, 195, 221
Anaciaeschna martini	Anisoptera	0.17	---	73	78	0.01	0	150, 188, 211, 214, 221
Anax imperator	Anisoptera	0.49	---	73.8	70.9	0	1	22, 32, 33, 75, 49, 63, 105, 110, 133,176, 179, 194, 195

Anax junius	Anisoptera	0.48	---	69.2	71.5	0	1	28, 157, 158, 195, 218
Anax parthenope	Anisoptera	0.46	---	75	74.5	0	1	13, 71, 110, 151, 178, 194, 195, 196, 199, 201, 211
Argia moesta	Zygoptera	---	0.31	40.8	40.2	0	1	25, 117, 118, 195, 218, 221
Argia vivida	Zygoptera	---	0.61	33.5	34	0	1	29, 34, 54, 65, 127, 195, 198, 218
Asiagomphus melaenops	Anisoptera	0.55	---	67	68.5	0	1	89, 210, 211, 221
Asiagomphus pryeri	Anisoptera	0.4	---	64.5	64.5	0	1	12, 13, 212, 221
Austrolestes colensonis	Zygoptera	0.52	---	44.6	---	0	1	34, 45, 193, 216
Boyeria irene	Anisoptera	0.47	---	63.1	61.5	0.03	0	50, 110, 133,192, 194
Brachythemis contaminata	Anisoptera	0.47	---	29.5	30.2	0.58	1	90, 107, 115, 122, 142, 143, 192, 195, 213, 221, 223
Calopteryx cornelia	Zygoptera	---	0.58	71.9	70	1	1	34, 133, 146, 199, 211, 221, 223
Calopteryx haemorrhoidalis	Zygoptera	---	0.58	47	43.1	0.89	1	20, 34, 39, 44, 110, 133, 194, 215, 223
Calopteryx maculata	Zygoptera	---	0.57	48.8	46.6	1	1	34, 53, 60, 76, 117, 133, 137, 166, 195, 198, 223
Calopteryx splendens	Zygoptera	---	0.52	45.9	43.9	0.81	1	34, 76, 131, 133, 176, 179,

								189, 191, 195, 223
Calopteryx virgo	Zygoptera	---	0.59	46.4	44.2	0.96	1	76, 82, 110, 133, 176, 179, 189, 194, 195, 223
Calopteryx xanthostoma	Zygoptera	---	0.75	45.7	44.3	0.8	1	110, 133, 176, 192, 194, 216, 223
Cercion lindenii	Zygoptera	---	0.61	33.2	32.3	0	1	34, 51, 164, 194, 195
Ceriagrion tenellum	Zygoptera	0.51	0.63	29.9	32.1	0	0	5, 6, 7, 8, 88, 110, 116, 119, 184, 194
Chalcolestes viridis	Zygoptera	0.52	0.58	44	40.8	0	1	3, 34, 36, 55, 75, 110, 133, 137, 194, 195
Chromagrion conditum	Zygoptera	---	0.87	34.8	33.7	0	0	23, 195, 198
Coenagrion hastulatum	Zygoptera	0.53	---	32	---	0	1	16, 75, 136, 189, 190, 194, 195, 204
Coenagrion mercuriale	Zygoptera	0.57	0.82	29.6	29.8	0	0	66, 110, 119, 129, 176, 194, 195
Coenagrion puella	Zygoptera	0.49	0.66	32.2	32.8	0	0	9, 11, 14, 34, 73, 78, 110, 128, 165, 179, 189, 194, 195
Coenagrion pulchellum	Zygoptera	---	0.68	34	35	0	0	165, 176, 179, 189, 194, 195
Coenagrion scitulum	Zygoptera	---	0.67	29	27.9	0	0	9, 110, 194, 195
Cordulegaster boltonii	Anisoptera	0.47	---	70.3	77.3	0	1	34, 50, 110, 133,

								176, 179, 194, 195
Cordulia aenea	Anisoptera	0.45	---	48.8	47.5	0	1	22, 34, 58, 75, 159, 160, 176, 179, 194, 221
Crocothemis erythraea	Anisoptera	0.55	---	38.5	35.4	0.06	1	2, 27, 60, 110, 133, 194, 195, 223
Deielia phaon	Anisoptera	0.41	---	42.5	41.5	0	1	91, 211, 221, 223
Diplacodes trivialis	Anisoptera	0.46	---	30.5	31.5	0.01	---	99, 107, 192, 195, 211, 213, 221, 223
Enallagma cyathigerum	Zygoptera	0.48	0.85	31.5	31.7	0	0	54, 97, 110, 114, 133,176, 179, 189, 194, 195
Epitheca bimaculata	Anisoptera	0.43	---	49.6	---	0.02	1	138, 179, 195, 208, 218, 223
Erythrodiplax umbrata	Anisoptera	---	0.17	42.5	---	0.56	1	100, 112, 181, 195, 205, 218, 223
Erythromma najas	Zygoptera	---	0.51	34.5	36.1	0	0	75, 165, 176, 179, 189, 195
Gomphus exilis	Anisoptera	0.48	---	43.3	40.8	0	0	96, 117, 133, 167, 181, 195, 198, 223
Gomphus flavipes	Anisoptera	0.46	---	46.5	49	0	0	21, 22, 33, 106, 198, 204, 207, 212
Gomphus pulchellus	Anisoptera	0.5	---	47.5	47.7	0	---	110, 144, 194, 195
Gomphus vulgatissimus	Anisoptera	0.47	---	47.2	48.8	0	1	22, 75, 79, 106, 110, 132, 136, 173, 176,

								179, 194, 195
Hetaerina americana	Zygoptera	---	0.8	44.6	38.1	0.1	1	30, 117, 118, 133, 134, 195, 198
Hetaerina vulnerata	Zygoptera	---	0.94	46.5	36	0.11	1	120, 121, 181, 195, 198
Ischnura cervula	Zygoptera	---	0.86	27.1	28.2	0	1	46, 65, 97, 117, 133, 195, 198, 218, 222, 223
Ischnura elegans	Zygoptera	0.51	0.57	30.3	30.5	0	0	1, 11, 43, 62, 73, 94, 95, 105, 110, 113, 132, 165, 176, 179, 195
Ischnura graellsii	Zygoptera	---	0.53	26.8	28	0	0	34, 35, 37, 38, 41, 42, 110, 192, 196
Ischnura posita	Zygoptera	---	0.51	24.1	23.8	0	0	125, 133, 135, 181, 195, 198, 222
Ischnura pumilio	Zygoptera	---	0.59	29.1	29.4	0	0	37, 40, 110, 176, 179, 189, 194, 195
Ladona depressa	Anisoptera	---	0.67	46.4	42	0.1	1	9, 34, 117, 133, 176, 179, 194, 195, 223
Ladona fulva	Anisoptera	---	0.77	43.6	42.6	0.03	1	24, 27, 133, 148, 176, 179, 194, 196, 223
Lestes barbarus	Zygoptera	---	0.59	40	37.6	0	0	110, 163, 194, 195
Lestes disjunctus	Zygoptera	---	0.63	36.8	37.4	0	0	10, 47, 124, 133, 137, 195,

								198, 218, 223
Lestes dryas	Zygoptera	0.45	---	37	36.2	0	0	73, 110, 176, 186, 189, 194, 195, 218
Lestes rectangularis	Zygoptera	0.36	---	43.2	40.2	0	0	56, 87, 181, 195, 198
Lestes sponsa	Zygoptera	0.46	0.68	35.4	37.9	0	0	73, 75, 110, 140, 141, 177, 189, 194, 195, 211, 221
Lestes virens	Zygoptera	---	0.65	34.7	33.8	0	0	110, 161, 163, 165, 194, 195
Leucorrhinia dubia	Anisoptera	0.46	---	37	35	0.02	1	28, 34, 75, 111, 176, 179, 194, 195, 221, 223
Leucorrhinia pectoralis	Anisoptera	0.46	---	35	---	0.01	1	22, 66, 174, 179, 194, 220, 223
Libellula pulchella	Anisoptera	---	0.61	52.6	49.7	0.33	1	34, 97, 98, 117, 133, 181, 195, 223
Libellula quadrimaculata	Anisoptera	0.47	---	43.5	43.1	0.08	1	34, 63, 75, 105, 110, 117, 118, 133, 176, 192, 194, 195, 211, 221, 223
Mnais pruinosa	Zygoptera	---	0.65	55.5	50	0.91	1	34, 108, 109, 133, 147, 149, 169, 199, 211, 221, 223
Nehalennia irene	Zygoptera	---	0.67	26.5	27.3	0	0	15, 69, 92, 186, 195,

								197, 198, 218
Onychogomphus forcipatus	Anisoptera	0.41	---	48.9	45.2	0	0	26, 61, 110, 133, 179, 194, 195
Onychogomphus uncatus	Anisoptera	0.46	---	51.6	48.5	0	1	27, 73, 74, 75, 81, 110, 123, 130, 133, 145, 212
Onychogomphus viridicostus	Anisoptera	0.49	---	58.4	---	0.02	1	22, 73, 185, 195, 198, 199
Ophiogomphus cecilia	Anisoptera	0.52	---	55	---	0	0	66, 103, 155, 171, 172, 179, 195, 204, 207
Orthetrum cancellatum	Anisoptera	0.45	---	48.5	47.8	0	1	34, 63, 75, 110, 133, 176, 179, 194, 195
Orthetrum coerulescens	Anisoptera	---	0.55	41.7	41	0	1	34, 74, 80, 110, 194, 195, 223
Orthetrum sabina	Anisoptera	0.47	---	55	55	0.01	1	34, 99, 107, 122, 142, 192, 195, 211, 213, 221, 223
Pantala flavescens	Anisoptera	0.47	---	48	49.5	0.03	1	34, 99, 107, 117, 118, 122, 142, 191, 195, 199, 205, 211, 213, 218, 221, 223
Paragomphus genei	Anisoptera	0.47	---	44.5	44.5	0	1	153, 194, 195, 198
Plathemis lydia	Anisoptera	---	0.56	41.5	39.8	0.49	1	34, 72, 85, 86, 117, 118, 192, 195, 221

Platycnemis pennipes	Zygoptera	---	0.54	37.1	36.4	0	0	27, 110, 133, 165, 176, 179, 189, 194, 195, 223
Pyrrhosoma nymphula	Zygoptera	0.52	0.68	37.1	36.4	0	1	17, 18, 31, 57, 86, 105, 110, 176, 189, 194, 195
Sieboldius albardae	Anisoptera	0.62	---	87.5	82.5	0	1	4, 13, 199, 209, 211, 221
Somatochlora alpestris	Anisoptera	0.66	---	47.2	---	0	1	83, 84, 179, 194, 195, 198, 223
Somatochlora arctica	Anisoptera	0.36	---	49	48.5	0	0	139, 175, 176, 179, 195, 223
Stylurus annulatus	Anisoptera	0.47	---	---	---	---	---	68, 221
Stylurus nagoyanus	Anisoptera	0.36	---	62	62	0	---	70, 211, 221
Stylurus oculatus	Anisoptera	0.4	---	---	---	1	1	68, 91, 209, 221
Sympecma fusca	Zygoptera	0.52	---	34.8	34.8	0	0	110, 156, 194, 195
Sympetrum danae	Anisoptera	---	0.49	31.2	32.2	0	0	102, 103, 192, 195, 198, 221, 223
Sympetrum depressiusculum	Anisoptera	---	0.63	33.9	---	0.01	0	101, 195, 221, 223
Sympetrum infuscatum	Anisoptera	---	0.61	42	45.5	0.14	1	170, 179, 195, 211, 221, 223
Sympetrum obtrusum	Anisoptera	---	0.34	34.5	27.6	0.02	0	52, 117, 168, 181, 192, 195, 223
Sympetrum pedemontanum	Anisoptera	---	0.49	35.5	35	0.22	0	110, 168, 194, 195, 199, 211, 221, 223
Sympetrum sanguineum	Anisoptera	0.46	---	34.7	35	0.02	1	48, 63, 64, 75, 104,

								110, 176, 179, 194, 195, 223
Sympetrum striolatum	Anisoptera	0.46	---	40.6	41.1	0.01	1	104, 110, 176, 179, 194, 195, 211, 221, 223
Sympetrum vicinum	Anisoptera	0.38	---	32.3	---	0.03	0	87, 117, 118, 190, 192, 195, 223
Sympetrum vulgatum	Anisoptera	0.46	---	33.7	32.5	0.01	1	27, 34, 63, 75, 110, 133, 179, 194, 223
Telebasis salva	Zygoptera	---	0.81	25.6	23.6	0	0	34, 97, 126, 133, 195, 198, 218, 223
Tetragoneuria cynosura	Anisoptera	0.47	---	---	---	0.23	1	28, 34, 86, 96, 183, 223
Trithemis annulata	Anisoptera	0.52	---	35.8	34.5	0.03	1	2, 110, 179, 180, 193, 195, 200, 223
Trithemis festiva	Anisoptera	0.51	---	34.5	33.5	0.02	1	99, 107, 122, 179, 182, 195, 213, 223
Xanthocnemis zealandica	Zygoptera	0.51	---	33	32.3	0	1	34, 45, 59, 193, 212

References

1. Abbott, J. K., & Svensson, E. I. (2008). Ontogeny of sexual dimorphism and phenotypic integration in heritable morphs. Evolutionary Ecology, 22, 103. https://doi.org/10.1007/s10682-007-9161-0
2. Agüero-Pelegrín, M., & Ferreras-Romero, M. (1994). Dragonfly emergence from an artificial pond in the urban area of Cordoba, Andalusia, southern Spain: A possible case of intraguild predation and competition between larvae. Notulae Odonatologicae, 4, 57-60.
3. Agüero-Pelegrín, M., Ferreras-Romero, M., & Corbet, P. S. (1999). The life cycle of *Lestes viridis* (Odonata: Lestidae) in two seasonal streams of the Sierra Morena Mountains (southern Spain). Aquatic Insects, 21, 187-196. https://doi.org/10.1076/aquin/21.3.187.4522

4. Aida, M. (1972). Observations on the emergence of *Sieboldius albardae* [In Japanese]. Gekkan Mushi, 15, 32-36.

5. Andrés, J. A. (1998). Polimorfismo y selección sexual en *Ceriagrion tenellum* (Odonata) (Doctoral dissertation). University of Vigo, Vigo, Spain.

6. Andrés, J. A., & Cordero-Rivera, A. (1998). Effects of water mites on the damselfly *Ceriagrion tenellum*. Ecological Entomology, 23, 103-109. https://doi.org/10.1046/j.1365-2311.1998.00125.x

7. Andrés, J. A., & Cordero-Rivera, A. (2001). Survival rates in a natural population of the damselfly *Ceriagrion tenellum*: effects of sex and female phenotype. Ecological Entomology, 26, 341-346. https://doi.org/10.1046/j.1365-2311.2001.00338.x

8. Andrés, J. A., Sánchez-Guillén, R. A., & Cordero-Rivera, A. (2002). Evolution of female colour polymorphism in damselflies: testing the hypotheses. Animal Behaviour, 63, 677-685. http://doi:10.1006/anbe.2001.1948

9. Angelibert, S., & Giani, N. (2003). Dispersal characteristics of three odonate species in a patchy habitat. Ecography, 26, 13-20. https://doi.org/10.1034/j.1600-0587.2003.03372.x

10. Anholt, B. R. (1997). Sexual size dimorphism and sex-specific survival in adults of the damselfly *Lestes disjunctus*. Ecological Entomology, 22, 127-132. https://doi.org/10.1046/j.1365-2311.1997.00055.x

11. Anholt, B. R., Vorburger, C., & Knaus, P. (2001). Mark-recapture estimates of daily survival rates of two damselflies (*Coenagrion puella* and *Ischnura elegans*). Canadian Journal of Zoology, 79, 895-899. https://doi.org/10.1139/z01-053

12. Aoki, T. (1999). Larval development, emergence and seasonal regulation in *Asiagomphus pryeri* (Selys) (Odonata: Gomphidae). Hydrobiologia, 394, 179-192. https://doi.org/10.1023/A:1003626011117

13. Bae, Y. J., & Lee, H. Y. 2012. Insect Fauna of Korea, Dragonflies (Arthropoda: Insecta: Odonata: Anisoptera: Gomphidae, Aeshnidae, Cordulegastridae). Republic of Korea: National Institute of Biological Resources, Junghaengsa Inc.

14. Banks, M. J., & Thompson, D. J. (1985). Emergence, longevity and breeding area fidelity in *Coenagrion puella* (L.) (Zygoptera: Coenagrionidae). Odonatologica, 14, 279-286.

15. Beatty, C. D., Andrés, J. A., & Sherratt, T. N. (2015). Conspicuous coloration in males of the damselfly *Nehalennia irene* (Zygoptera: Coenagrionidae): do males signal their unprofitability to other males? PLoS One, 10, e0142684. https://doi.org/10.1371/journal.pone.0142684

16. Befeld, S., Katzur, K., Lepkojus, S., & Rolff, J. (1997). Emergence patterns of *Coenagrion hastulatum* (Charpentier) in northern Germany (Zygoptera: Coenagrionidae). Odonatologica, 26, 337-342.

17. Bennett, S., & Mill, P. J. (1995). Lifetime egg production and egg mortality in the damselfly *Pyrrhosoma nymphula* (Sulzer) (Zygoptera: Coenagrionidae). Hydrobiologia, 310, 71-78. https://doi.org/10.1007/BF00008184

18. Bennett, S., & Mill, P. J. (1995). Pre-and post-maturation survival in adults of the damselfly *Pyrrhosoma nymphula* (Zygoptera: Coenagrionidae). Journal of Zoology, 235, 559-575. https://doi.org/10.1111/j.1469-7998.1995.tb01768.x

19. Bernard, R., & Samolag, J. (1997). Analysis of the emergence of *Aeshna affinis* (Vander Linden, 1823) in the vicinity of Poznań, western Poland (Odonata: Aeshnidae). Opuscula Zoologica Fluminense, 153: 1-12.

20. Beukema, J. J. (2002). Survival rates, site fidelity and homing ability in territorial *Calopteryx haemorrhoidalis* (Vander Linden) (Zygoptera: Calopterygidae). Odonatologica, 31, 9-22.

21. Beutler, H. (1985). Beitrag zur Fotodokumentation heimischer Libellen: *Gomphus flavipes* Charp. [Contribution to the photo-documentation of native dragonflies: *Gomphus flavipes* Charp.]. Libellula, 4, 158-159.

22. Beutler, H. (1986). Zur Schlupfrate und zum Geschlechterverhältnis einheimischer Grosslibellen (Anisoptera) (Odonata) [The hatching rate and the sex ratio of native dragonflies (Anisoptera) (Odonata)]. Entomologische Abhandlungen Staatliches Museum für Tierkunde Dresden, 49, 201-209.
23. Bick, G. H., Bick, J. C., & Hornuff, L. E. (1976). Behavior of *Chromagrion conditum* (Hagen) adults (Zygoptera: Coenagrionidae). Odonatologica, 5, 129-141.
24. Boano, G., & Rolando, A. (2003). Aggressive interactions and demographic parameters in *Libellula fulva* (Odonata, Libellulidae). Italian Journal of Zoology, 70, 159-166. https://doi.org/10.1080/11250000309356510
25. Borror, D. J. (1934). Ecological studies of *Argia moesta* Hagen (Odonta: Coenagrionidae) by Means of Marking. Ohio Journal of Science, 34, 97-108.
26. Brendel, U. (1992). Die Ansprüche der Kleinen Zanglibelle (*Onychogomphus forcipatus*) an das Biotop Fliessgewässer (Odonata: Gomphidae) [The claims of the Little Zanglihelle (*Onychogomphus forcipatus*) to the biotope Riverside (Odonata: Gomphidae)] (Doctoral dissertation). University of Regensburg, Regensburg, Germany.
27. Brooks, S., Cham, S., & Lewington, R. (1997). Field guide to the dragonflies and damselflies of Great Britain and Ireland. British Wildlife Publishing.
28. Campanella, P. J. (1975). The evolution of mating systems in temperate zone dragonflies (Odonata: Anisoptera) II: *Libellula luctuosa* (Burmeister). Behaviour, 54, 278-310. https://doi.org/10.1163/156853975X00281
29. Conrad, K. F., & Pritchard, G. (1988). The reproductive behavior of *Argia vivida* Hagen: an example of a female-control mating system (Zygoptera: Coenagrionidae). Odonatologica, 17, 179-185.
30. Contreras-Garduno, J., Buzatto, B. A., Serrano-Meneses, M. A., Nájera-Cordero, K., & Córdoba-Aguilar, A. (2008). The size of the red wing spot of the American rubyspot as a heightened condition-dependent ornament. Behavioral Ecology, 19, 724-732. https://doi.org/10.1093/beheco/arn026
31. Corbet, P. S. (1952). An adult population study of *Pyrrhosoma nymphula* (Sulzer):(Odonata: Coenagrionidae). The Journal of Animal Ecology, 21, 206-222. http://dio.org/ 10.2307/1958
32. Corbet, P. S. (1957). The life-history of the emperor dragonfly *Anax imperator* Leach (Odonata: Aeshnidae). The Journal of Animal Ecology, 26, 1-69. http://doi.org/ 10.2307/1781
33. Corbet, P. S. (1962). A biology of dragonflies. London: HF & G. Witherby LTD.
34. Corbet, P. S. (1999). Dragonflies: behaviour and ecology of Odonata. Colchester, United Kingdom: Harley Books.
35. Cordero-Rivera, A. (1987). Estructura de población en *Ischnura graellsii* Rambur, 1842 (Zygop. Coenagrionidae) [Population structure in *Ischnura graellsii* Rambur, 1842 (Zygop. Coenagrionidae)]. Boletín de la Asociación Española de Entomología, 11, 269-286.
36. Cordero-Rivera, A. (1988). Estudio ecológico de una población de *Lestes viridis* Vander Linden, 1825 (Zygoptera, Lestidae) [Ecological study of a population of *Lestes viridis* Vander Linden, 1825 (Zygoptera, Lestidae)]. Limnética, 4, 1-8.
37. Cordero-Rivera, A. (1992). Density-dependent mating success and colour polymorphism in females of the damselfly *Ischnura graellsii* (Odonata: Coenagrionidae). Journal of Animal Ecology, 61, 769-780. http://doi.org/ 10.2307/5630
38. Cordero-Rivera, A. (1995). Correlates of male mating success in two natural populations of the damselfly *Ischnura graellsii* (Odonata: Coenagrionidae). Ecological Entomology, 20, 213-222. https://doi.org/10.1111/j.1365-2311.1995.tb00450.x
39. Cordero-Rivera, A. (1999). Forced copulations and female contact guarding at a high male density in a calopterygid damselfly. Journal of Insect Behavior, 12, 27-37. https://doi.org/10.1023/A:1020972913683
40. Cordero-Rivera, A., & Andrés, J. A. (1999). Lifetime mating success, survivorship and synchronized reproduction in the damselfly *Ischnura pumilio* (Odonata: Coenagrionidae). International Journal of Odonatology, 2, 105-114.

41. Cordero-Rivera, A., & Egidio-Pérez, F. J. (1998). Mating frequency, population density and female polychromatism in the damselfly *Ischnura graellsii*: an analysis of four natural populations. Etología, 6, 61-67.
42. Cordero-Rivera, A., Egidio-Perez, F. J., & Andres, J. A. (2002). The effect of handling damage, mobility, body size, and fluctuating asymmetry on lifetime mating success of *Ischnura graellsii* (Rambur) (Zygoptera: Coenagrionidae). Odonatologica, 31, 117-128.
43. Cordero-Rivera, A., Santolamazza-Carbone, S., & Utzeri, C. (1998). Mating opportunities and mating costs are reduced in androchrome female damselflies, *Ischnura elegans* (Odonata). Animal Behaviour, 55, 185-197. https://doi.org/10.1006/anbe.1997.0603
44. Córdoba-Aguilar, A. (2002). Wing pigmentation in territorial male damselflies, *Calopteryx haemorrhoidalis*: a possible relation to sexual selection. Animal Behaviour, 63, 759-766. https://doi.org/10.1006/anbe.2001.1974
45. Deacon, K. J. (1979). The seasonality of four Odonata species from mid Canterbury, South Island, New Zealand (Doctoral dissertation). University of Canterbury, Canterbury, New Zealand. https://ir.canterbury.ac.nz/handle/10092/6958
46. Dickerson, J. E., Tyler, T., & Robinson, J. V. (1992). Aspects of the life-history of adult *Ischnura cervula* (Selys) (Zygoptera: Coenagrionidae). Notulae Odonatologicae, 3, 137-139.
47. Duffy, W. G. (1994). Demographics of *Lestes disjunctus disjunctus* (Odonata: Zygoptera) in a riverine wetland. Canadian Journal of Zoology, 72, 910-917. https://doi.org/10.1139/z94-124
48. Falck, J., & Johansson, F. (2000). Patterns in size, sex ratio and time at emergence in a south Swedish population of *Sympetrum sanguineum* (Odonata). Aquatic Insects, 22, 311-317. https://doi.org/10.1076/0165-0424(200010)22:4;1-Y;FT311
49. Fastenrath, V. H. 1950. Massenschlüfen von *Anax imperator* [Mass testing of *Anax imperator*]. Westdeutscher Naturwart, Boon, 1, 22-23.
50. Ferreras-Romero, M., & Corbet, P. S. (1995). Seasonal patterns of emergence in Odonata of a permanent stream in southwestern Europe. Aquatic Insects, 17, 123-127. https://doi.org/10.1080/01650429509361580
51. Fincke, O. M. (1986). Lifetime reproductive success and the opportunity for selection in a nonterritorial damselfly (Odonata: Coenagrionidae). Evolution, 40, 791-803. https://doi.org/10.1111/j.1558-5646.1986.tb00539.x
52. Forbes, M. R., Muma, K. E., & Smith, B. P. (2002). Recapture of male and female dragonflies in relation to parasitism by mites, time of season, wing length and wing cell symmetry. Experimental & Applied Acarology, 34, 79-93. https://doi.org/10.1023/B:APPA.0000044441.60122.27
53. Forsyth, A., & Montgomerie, R. D. (1987). Alternative reproductive tactics in the territorial damselfly *Calopteryx maculata*: sneaking by older males. Behavioral Ecology and Sociobiology, 21, 73-81. https://doi.org/10.1007/BF02395434
54. Garrison, R. W. (1978). A mark-recapture study of imaginal *Enallagma cyathigerum* (Charpentier) and *Argia vivida* Hagen (Zygoptera: Coenagrionidae). Odonatologica, 7, 223-236.
55. Geenen, S., Jordaens, K., De Block, M., Stoks, R., & De Bruyn, L. (2000). Genetic differentiation and dispersal among populations of the damselfly *Lestes viridis* (Odonata). Journal of the North American Benthological Society, 19, 321-328. https://doi.org/10.2307/1468074
56. Gower, J. L., & Kormondy, E. J. (1963). Life history of damselfly *Lestes rectangularis* with special reference to seasonal regulation. Ecology, 44, 398-402. http://doi.org/ 10.2307/1932189
57. Gribbin, S. D., & Thompson, D. J. (1991). The effects of size and residency on territorial disputes and short-term mating success in the damselfly *Pyrrhosoma nymphula* (Sulzer) (Zygoptera: Coenagrionidae). Animal Behaviour, 41, 689-695. https://doi.org/10.1016/S0003-3472(05)80906-6
58. Ha, L. Y., Wildermuth, H., & Dorn, S. (2002). Emergenz von *Cordulia aenea* (Odonata: Corduliidae) [Emergence of *Cordulia aenea* (Odonata: Corduliidae)]. Libellula, 21, 1-14.

59. Hardersen, S. (2000). Effects of carbaryl exposure on the last larval instar of *Xanthocnemis zealandica*– fluctuating asymmetry and adult emergence. Entomologia Experimentalis et Applicata, 96, 221-230. https://doi.org/10.1046/j.1570-7458.2000.00700.x

60. Hassan, A. T. (1978). Reproductive behaviour of *Acisoma panorpoides inflatum* Selys (Anisoptera: Libellulidae). Odonatologica, 7, 237-245.

61. Herden, K. (1990). Einige Untersuchungen zur Biologie von *Onychogomphus forcipatus* L. (Odonata: Gomphidae) [Some biology studies of *Onychogomphus forcipatus* L. (Odonata: Gomphidae)] (Doctoral dissertation). Albert Ludwig University of Freiburg, Freiburg im Breisgau, Baden-Württemberg, Germany.

62. Hinnekint, B. O. N. (1987). Population dynamics of *Ischnura E. elegans* (Vander Linden) (Insecta: Odonata) with special reference to morphological colour changes, female polymorphism, multiannual cycles and their influence on behaviour. Hydrobiologia, 146, 3-31. https://doi.org/10.1007/BF00007574

63. Hoesss, R. (1993). Die aquatische invertebratenfauna im Naturschutzgebiet Auried (Kieinhösingen, FR) [The aquatic invertebrate fauna in the nature reserve Auried (Kieinhösingen, FR)] (Bachelor thesis). University of Bern, Bern, Germany.

64. Hofmann, B., & Martens, A. (2005). Eine fang-wiederfang-studie zur Ortstreue und Kurzstreckenausbreitung von *Sympetrum sanguineum* (Odonata: Libellulidae) [A catch-repeat study of local fidelity and short-range dispersal of *Sympetrum sanguineum* (Odonata: Libellulidae)]. Libellula, 24, 63-72.

65. Hornung, C. L., & Pacas, C. (2006). Investigating damselfly populations at springs in Banff National Park, Canada, with special focus on *Argia vivida*, *Amphiagrion abbreviatum*, and *Ischnura cervula* (Odonata: Coenagrionidae). Aquatic Ecology, 40, 49-58. https://doi.org/10.1007/s10452-005-9017-z

66. Hunger, H. (2004). Naturschutzorientierte, GIS-Gestützte Untersuchungen zur Bestandssituation der Libellenarten *Coenagrion mercuriale*, *Leucorrhinia pectoralis* und *Ophiogomphus cecilia* (Anhang II FFH-Richtlinie) in Baden-Württemberg [Conservation-oriented, GIS-based investigations on the status of the dragonfly species *Coenagrion mercuriale*, *Leucorrhinia pectoralis* and *Ophiogomphus cecilia* (Annex II FFH-Directive) in Baden-Württemberg] (Doctoral dissertation). Albert Ludwig University of Freiburg, Freiburg im Breisgau, Baden-Württemberg, Germany. https://www.inula.de/referenzen/veroeffentlichungen.html

67. Inden-Lohmar, C. (1997). Sukzession, Struktur und Dynamik von Libellenpopulationen an Kleingewässern, unter besonderer Berücksichtigung der Ökologie von *Aeshna cyanea* (Müller, 1764) [Succession, structure and dynamics of dragonfly populations on ponds, with special consideration of the ecology of *Aeshna cyanea* (Müller, 1764)] (Doctoral dissertation). Rheinische Friedrich-Wilhelms-University of Bonn, Bonn, Germany.

68. Inoue, K. (1979). Review of Japanese dragonflies. Part 6 (concluded). Families Coenagrionidae, Platycnemididae, Lestidae and Megapodagrionidae [In Japanese]. Gracile, 24, 1-20.

69. Iserbyt, A., Bots, J., Ting, J. J., Jvostov, F. P., Forbes, M. R., Sherratt, T. N., & Van Gossum, H. (2009). Multi-annual variation in female morph frequencies of the polymorphic damselfly, *Nehalennia irene*, at continental & regional scales. Animal Biology, 59, 313–326. https://doi.org/10.1163/157075609X454944

70. Ishida, M. (1982). Observations of the emergence of *Stylurus nagoyanus* at Niigata [In Japanese]. Konchu to shizen [Nature & Insects], 17, 28-29.

71. Itô. Y. 1981. Comparative ecology. Cambridge, United Kingdom: Cambridge University Press.

72. Jacobs, M. E. (1955). Studies on territorialism and sexual selection in dragonflies. Ecology, 36, 566-586. https://doi.org/10.2307/1931296

73. Jahn, A. (1991). Ökologische untersuchungen über die fauna einer baugrube des Wendlands unter besonderer berücksichtigung der libellen. [Ecological research on the fauna of a pit of the Wendland with special regard to dragonflies] (Thesis). University of Hamburg.

74. Jakob, C. (1995). Emergenz von *Onychogomphus uncatus* Charpentier 1840 (Odonata: Gomphidae) und *Orthetrum coerulescens* Fabricius 1798 (Odonata: Libellulidae) unter besonderer Berücksichtigung der Mortalität beim Schlupf [Emergence of *Onychogomphus uncatus* Charpentier 1840 (Odonata: Gomphidae)

and *Orthetrum coerulescens* Fabricius 1798 (Odonata: Libellulidae) with special consideration of mortality during hatching] (Doctoral dissertation). University of Heidelberg, Heidelberg, Baden-Württemberg, Germany.

75. Johansson, F., Crowley, P. H., & Brodin, T. (2005). Sexual size dimorphism and sex ratios in dragonflies (Odonata). Biological Journal of the Linnean Society, 86, 507-513. https://doi.org/10.1111/j.1095-8312.2005.00549.x
76. Johnson, C. (1964). The evolution of territoriality in the Odonata. Evolution, 18, 89-92. http://doi.org/10.2307/2406422
77. Kaiser, H. (1985). Availability of receptive females at the mating place and mating chances of males in the dragonfly *Aeschna cyanea*. Behavioral Ecology and Sociobiology, 18, 1-7. https://doi.org/10.1007/BF00299231
78. Karaman, B. S. (1987). Le sex-ratio chez une espèce de zygoptère, *Coenagrion puella* (L.) (Odonata, Coenagrionidae) [The sex ratio in a zygopteran species, *Coenagrion puella* (L.) (Odonata, Coenagrionidae)]. Godisen Zboruik Priroduo-Matematicki Fakultet na Universitetot Skopje (Biologija) [Annual Compendium of Natural Sciences and Mathematics], 38, 163–173.
79. Kern, D. (1992). Beobachtungen an *Gomphus vulgatissimus* (L.) an einem Wiesengraben der Dümmer-Geestniederung (Anisoptera: Gomphidae) [Observations on *Gomphus vulgatissimus* (L.) on a meadow trench of the Dümmer-Geest lowlands (Anisoptera: Gomphidae)]. Libellula, 11, 47-76.
80. Kéry, M., & Juillerat, L. (2004). Sex ratio estimation and survival analysis for *Orthetrum coerulescens* (Odonata, Libellulidae). Canadian Journal of Zoology, 82, 399-406. https://doi.org/10.1139/z04-004
81. Kleemeyer, A. (1994). EinfluB der larvengroBe auf korpermaBe der imagines von *Onychogomphus uncatus* (Charpentier, 1840) (Odonata: Gomphidae) [Influence of the larvae on the body of the imagines of *Onychogomphus uncatus* (Charpentier, 1840) (Odonata: Gomphidae)] (Doctoral dissertation). University of Braunschweig-Institute of Technology, Braunschweig, Lower Saxony, Germany.
82. Klötzli, A. M. (1971). Zur revierstetigkeit von *Calopteryx virgo* (L.) [On the revolutions of *Calopteryx virgo* (L.)]. Mitteilungen der Schweizerischen Enttomologischen Gesellschaft, 43, 240-248.
83. Knaus, P. (1999). Untersuchungen zur Emergenz, zur Mobilität und zum Paarungssystem an einer Metapopulation von *Somatochlora alpestris* (Selys 1840) in den Zentralalpen (Anisoptera: Corduliidae) [Studies on the emergence, mobility and mating system of a metapopulation of *Somatochlora alpestris* (Selys 1840) in the Central Alps (Anisoptera: Corduliidae)] (Doctoral dissertation). University of Zurich, Zurich, Switzerland.
84. Knaus, P., & Wildermuth, H. (2002). Site attachment and displacement of adults in two alpine metapopulations of *Somatochlora alpestris* (Odonata: Corduliidae). International Journal of Odonatology, 5, 111-128. https://doi.org/10.1080/13887890.2002.9748183
85. Koenig, W. D., & Albano, S. S. (1987). Lifetime reproductive success, selection, and the opportunity for selection in the white-tailed skimmer *Plathemis lydia* (Odonata: Libellulidae). Evolution, 41, 22-36. https://doi.org/10.1111/j.1558-5646.1987.tb05768.x
86. Kormondy, E. J. (1961). Territoriality and dispersal in dragonflies (Odonata). Journal of the New York Entomological Society, 69, 42-52.
87. Kormondy, E. J., & Gower, J. L. (1965). Life history variations in an association of Odonata. Ecology, 46, 882-886. https://doi.org/10.2307/1934027
88. Krüner, U. (1989). Die Verbreitung, biologie und ökologie der späten adonislibelle *Ceriagion tenellum* (Odonata, Coenagrionidae) in Nordwesteuropa, insbesondere im Gebiet zwischen Maas und Rhein [The distribution, biology and ecology of the late Adonislibelle *Ceriagion tenellum* (Odonata, Coenagrionidae) in northwestern Europe, especially in the area between the Meuse and the Rhine]. Verhandlungen Westdeutscher Entomologen, 1988, 133-140.
89. Kurata, M. (1971). The life history of *Gomphus melaenops* (Gomphidae) [In Japanese]. Tombo, 14, 6-11.

90. Kurata, M., & Morozumi, T. (1966). Some ecological observations on the emergence of *Ictinogomphus clavatus* Fabricius (Anisoptera, Gomphidae) [In Japanese]. Tombo, 9, 17-22.
91. Kurata, M., & Morozumi, T. (1969). Fauna & ecological observations of dragonflies from Lake Suwako. Nagano Prefecture, central Japan [In Japanese]. Atarashī konchū-gaku jānaru [New Entomologist]. 18, 53-60.
92. Lajeunesse, M. J., & Forbes, M. R. (2003). A comparison of structural size and condition in two female morphs of the damselfly *Nehalennia irene* (Hagen) (Zygoptera: Coenagrionidae). Odonatologica, 32, 281-287.
93. Leyshon, O. J., & Moore, N. W. (1993). A note on the British Dragonfly Society's survey of *Anaciaeschna isosceles* at Castle Marshes, Barnby, Suffolk, 1991-1992. Journal of the British Dragonfly Society, 9, 5-9.
94. Lord, P. M. (1961). A study of the colour varieties of some damselflies (Doctoral dissertation). University of Wales, Wales, United Kingdom.
95. Lösing, U. (1988). Auswertung faunistisch-ökologischer Bestandsaufnahmen in NSG Achmer Grasmoor und der geplanten Erweiterungsfläche im Hinblick auf Pflege und Entwicklung [Evaluation of faunistic-ecological inventories in NSG Achmer Grasmoor and the planned expansion area with regard to care and development] (Master thesis). Paderbom University, North Rhine-Westphalia, Germany.
96. Lutz, P. E., & McMahan, E. A. (1973). Five-year patterns of emergence in *Tetragoneuria cynosura* and *Gomphus exilis* (Odonata). Annals of the Entomological Society of America, 66, 1343-1348. https://doi.org/10.1093/aesa/66.6.1343
97. Manolis, T. (2003). Dragonflies and damselflies of California. University of California Press.
98. Marden, J. H., & Rowan, B. (2000). Growth, differential survival, and shifting sex ratio of free-living *Libellula pulchella* (Odonata: Libellulidae) dragonflies during adult maturation. Annals of the Entomological Society of America, 93, 452-458. https://doi.org/10.1603/0013-8746(2000)093[0452:GDSASS]2.0.CO;2
99. Mathavan, S., & Pandian, T. J. (1977). Patterns of emergence, import of egg energy and energy export via emerging dragonfly populations in a tropical pond. Hydrobiologia, 54, 257-272. https://doi.org/10.1007/BF00014293
100. Meurgey, F., David, G., & Thiebaut, B. (2008). Collecting trip in Martinique (French West Indies). Argia, 20, 14-16.
101. Michiels, N. K., & Dhondt, A. A. (1987). Coexistence of three *Sympetrum* species at Den Diel, Mol, Belgium (Anisoptera: Libellulidae). Odonatologica, 16, 347-360.
102. Michiels, N. K., & Dhondt, A. A. (1989). Effects of emergence characteristics on longevity and maturation in the dragonfly *Sympetrum danae* (Anisoptera: Libellulidae). Hydrobiologia, 171, 149-158. https://doi.org/10.1007/BF00008175
103. Michiels, N. K., & Dhondt, A. A. (1991). Characteristics of dispersal in sexually mature dragonflies. Ecological Entomology, 16, 449-459. https://doi.org/10.1111/j.1365-2311.1991.tb00238.x
104. Moore, N. W. (1952). On the so-called" territories" of dragonflies (Odonata-Anisoptera). Behaviour, 4, 85-100. https://doi.org/10.1163/156853951X00052
105. Moore, N. W. (1964). Intra-and interspecific competition among dragonflies (Odonata). The Journal of Animal Ecology, 33, 49-71. http://doi.org/ 10.2307/2348
106. Müller, O. (1995). Ökologische Untersuchungen an Gomphiden (Odonata: Gomphidae) unter besonderer Berücksichtigung ihrer Larvenstadien [Ecological investigations on gomphids (Odonata: Gomphidae) with special consideration of their larval stages] (Doctoral dissertation). Humboldt University, Berlin, Germany.
107. Nair, M. V. (2011). Dragonflies & damselflies of Orissa and Eastern India. Orissa, Neelakanta Nagar, Bhubaneswar, India: Wildlife Organization, Forest & Environment Department, Government of Orissa.
108. Nomakuchi, S., Higashi, K., & Maeda, M. (1988). Synchronization of reproductive period among the two male forms and female of the damselfly *Mnais pruinosa* Selys (Zygoptera: Calopterygidae). Ecological Research, 3, 75-87. https://doi.org/10.1007/BF02346931

109. Nomakuchi, S., Higashi, K., Harada, M., & Maeda, M. (1984). An experimental study of the territoriality in *Mnais pruinosa pruinosa* Selys (Zygoptera: Calopterygidae). Odonatologica, 13, 259-267.
110. Ocharán, F. J. (1987). Los Odonatos de Asturias y de España. Aspectos sistemáticos y faunísticos [The Odonatos of Asturias and Spain. Systematic and faunal aspects] (Doctoral dissertation), University of Oviedo, Oviedo, Spain. http://digibuo.uniovi.es/dspace/handle/10651/16892
111. Pajunen, V. I. (1962). Studies on the population ecology of *Leucorrhinia dubia* VD Lind. (Odon., Libellulidae). Annales Zoologici Societatis Zoologicae Fennicae Vanamo, 24, 1-69.
112. Palacino-Rodríguez, F., Contreras-Sánchez, N. A., & Córdoba-Aguilar, A. (2012). Population structure in dry and rainy seasons in *Erythrodiplax umbrata* (Linnaeus, 1758) (Odonata: Libellulidae). Odonatologica, 41, 245-249.
113. Parr, M. J. (1973). Ecological studies of *Ischnura elegans* (Vander Linden) (Zygoptera: Coenagrionidae). II. Survivorship, local movements and dispersal. Odonatologica, 2, 159-174.
114. Parr, M. J. (1976). Some aspects of the population ecology of the damselfly *Enallagma cyathigerum* (Charpentier) (Zygoptera: Coenagrionidae). Odonatologica, 5, 45-57.
115. Parr, M. J. (1983). An analysis of territoriality in libellulid dragonflies (Anisoptera: Libellulidae). Odonatologica, 12, 39-57.
116. Parr, M. J., & Parr, M. (1979). Some observations on *Ceriagrion tenellum* (de Villers) in southern England (Zygoptera: Coenagrionidae). Odonatologica, 8, 171-194.
117. Paulson, D. (2009). Dragonflies and damselflies of the West. Princeton University Press.
118. Paulson, D. (2011). Dragonflies and damselflies of the East. Princeton University Press.
119. Purse, B. V., & Thompson, D. J. (2003). Emergence of the damselflies, *Coenagrion mercuriale* and *Ceriagrion tenellum* (Odonata: Coenagrionidae), at their northern range margins, in Britain. European Journal of Entomology, 100, 93-100.
120. Ramírez-Delgado, J. 2016. Caracterización de las fibras del músculo de vuelo en machos territoriales y no territoriales de *Hetaerina vulnerata* [Characterization of flight muscle fibers in territorial and non-territorial males of Hetaerina vulnerata]. (Master thesis). Autonomous University of Tlaxcala, Tlaxcala, México.
121. Ramírez-Delgado, J., López-García, K., Lara, C., & Serrano-Meneses, M. A. (2015). Wing pigmentation in males of a territorial damselfly: alternative reproductive tactics, allometry and mating success. Journal of Insect Behavior, 28, 569-581. https://doi.org/10.1007/s10905-015-9528-y
122. Rawat, M., Dookia, S., & Sivaperuman, C. (2015). Aquatic ecosystem: Biodiversity, ecology and conservation. New Delhi, India: Springer.
123. Rehfeldt, G. (2004). Diel pattern of activity, mating, and flight behaviour in *Onychogomphus uncatus* (Odonata: Gomphidae). International Journal of Odonatology, 7, 65-71. https://doi.org/10.1080/13887890.2004.9748195
124. Robb, T., Van Gossum, H., & Forbes, M. R. (2006). Colour variation in female *Lestes disjunctus* Selys: a second example of a polymorphic lestid (Zygoptera: Lestidae). Odonatologica, 35, 31-39.
125. Robinson, J. V. (1983). Effects of water mite parasitism on the demographics of an adult population of *Ischnura posita* (Hagen) (Odonata: Coenagrionidae). American Midland Naturalist, 109, 169-174. http://doi.org/ 10.2307/2425527
126. Robinson, J. V., & Frye, B. L. (1986). Survivorship, mating and activity pattern of adult *Telebasis salva* (Hagen) (Zygoptera: Coenagrionidae). Odonatologica, 15, 211-217.
127. Robinson, J. V., Dickerson, J. E., & Bible, D. R. (1983). The demographics and habitat utilization of adult *Argia sedula* (Hagen) as determined by mark-recapture analysis (Zygoptera: Coenagrionidae). Odonatologica, 12, 167-172.
128. Rolff, J., Antvogel, H., & Schrimpf, I. (2000). No correlation between ectoparasitism and male mating success in a damselfly: why parasite behavior matters. Journal of Insect Behavior, 13, 563-571. https://doi.org/10.1023/A:1007867719887

129. Rouquette, J. R., & Thompson, D. J. (2005). Habitat associations of the endangered damselfly, *Coenagrion mercuriale*, in a water meadow ditch system in southern England. Biological Conservation, 123, 225-235. https://doi.org/10.1016/j.biocon.2004.11.011

130. Schütte, C. (1992). Entwicklung und Populationsstruktur von *Onychogomphus uncatus* (Charpentier, 1840) (Odonata: Gomphidae) [Development and population structure of *Onychogomphus uncatus* (Charpentier, 1840) (Odonata: Gomphidae)] (Doctoral dissertation). University of Braunschweig-Institute of Technology, Braunschweig, Lower Saxony, Germany.

131. Schutte, G., Reich, M., & Plachter, H. (1997). Mobility of the rheobiont damselfly *Calopteryx splendens* (Harris) in fragmented habitats (Zygoptera: Calopterygidae). Odonatologica, 26, 317-327.

132. Schwaller, T., & K. Eigenheer. 1989. Die libellenfauna im Bezirk Wasseramt, Kanton Solothurn, Schweiz (Odonata) [The dragonfly fauna in the district Wasseramt, Canton Solothurn, Switzerland (Odonata)]. Opuscula Zoologica Fluminensia, 34, 1-32

133. Serrano-Meneses, M. A., Córdoba-Aguilar, A., Azpilicueta-Amorín, M., González-Soriano, E., & Székely, T. (2008). Sexual selection, sexual size dimorphism and Rensch’s rule in Odonata. Journal of Evolutionary Biology, 21, 1259-1273. https://doi.org/10.1111/j.1420-9101.2008.01567.x

134. Serrano-Meneses, M. A., Córdoba-Aguilar, A., Méndez, V., Layen, S. J., & Székely, T. (2007). Sexual size dimorphism in the American rubyspot: male body size predicts male competition and mating success. Animal Behaviour, 73, 987-997. https://doi.org/10.1016/j.anbehav.2006.08.012

135. Shaffer, L. R., & Robinson, J. V. (1989). Sex ratio and sexual dimorphism in late instar larvae of *Ischnura posita* (Hagen) (Zygoptera: Coenagrionidae). Notulae Odonatologicae, 3, 40-41.

136. Smallshire, D., & Swash, A. (2014). Britain's dragonflies: A field guide to the damselflies and dragonflies of Britain and Ireland-Fully revised and updated. Princeton University Press.

137. Sokolovska, N., Rowe, L., & Johansson, F. (2000). Fitness and body size in mature odonates. Ecological Entomology, 25, 239-248. https://doi.org/10.1046/j.1365-2311.2000.00251.x

138. Sonehara, I. (1982). Life history of *Epitheca bimaculata sibirica* at Mount Yatsugatake [In Japanese]. Nagano, Japan: Sinano Kyoikukai Corporation.

139. Sonehara, I. (1985). Observations on the life-history of *Somatochlora arctica* in Nagano Prefecture [In Japanese]. Tombo, 28, 23-30.

140. Stoks, R. (2001). Male-biased sex ratios in mature damselfly populations: real or artefac? Ecological Entomology, 26, 181-187. https://doi.org/10.1046/j.1365-2311.2001.00301.x

141. Stoks, R. (2001). What causes male-biased sex ratios in mature damselfly y populations? Ecological Entomology, 26, 188-197. https://doi.org/10.1046/j.1365-2311.2001.00303.x

142. Subramanian, K. A. (2005). Dragonflies & damselflies of peninsular India- Afield guide. Bangalore. India: Centre for Ecological Science, Indian Institute of Science & Indian Academy of Sciences. E-Book of Project Lifescape.

143. Subramanian, K. A. (2009). Dragonflies of India. A field guide. New Delhi, India: Indian Offset Press. REVISAR; PAGA PARA MAS ESPECIES

144. Suhling, F. (1991). Habitatansprüche der Larven von *Gomphus pulchellus* Sélys, 1840 (Odonata: gomphidae) [Habitat claims of the larvae of *Gomphus pulchellus* Sélys, 1840 (Odonata: gomphidae)] (Doctoral dissertation). University of Braunschweig-Institute of Technology, Braunschweig, Lower Saxony, Germany.

145. Suhling, F. (1995). Temporal patterns of emergence of the riverine dragonfly *Onychogomphus uncatus* (Odonata: Gomphidae). Hydrobiologia, 302, 113-118. https://doi.org/10.1007/BF00027036

146. Suzuki, K., & Tamaishi, A. (1981). Ethological study on *Calopteryx cornelia* Selys (Odonata, Calopterygidae). I. Analysis of adult behavior by marking-reobservation experiments. The Journal of College of Liberal Arts, Toyama University, Natural Science, 14, 25-40.

147. Suzuki, K., & Tamaishi, A. (1982). Ethological study on two *Mnais* species, *Mnais nawai* Yamamoto and *M. pruinosa* Selys, in the Hokuriku District, central Honshu, Japan. Analysis of adult behavior by marking-

reobservation experiments. The Journal of College of Liberal Arts, Toyama University, Natural Science, 14, 95-128.

148. Szállassy, N., Bárdosi, E., Szabó, Z. D., Szép, T., & Dévai, G. (2003). Fluctuating asymmetry, survival and mating success in male *Libellula fulva* Mueller (Anisoptera: Libellulidae). Odonatologica, 32, 143-151.
149. Taguchi, M., & Watanabe, M. (1993). Life history of the larvae of *Mnais pruinosa costalis* (Odonata, Calopterygidae). Japanese Journal of Entomology, 61, 371-376.
150. Taketo, A. (1994). Seasonal change of emergence & sex ratio of *Anaciaeschna martini* Selys [In Japanese]. Tombo, 37, 47-48.
151. Taketo, A. (1995). Emergence pattern and sex ratio of four aeschnid dragonflies in newly formed ponds [In Japanese]. Tombo, 38, 48-50.
152. Taylor, M. (2013). Dragonflight: In Search of Britain's dragonflies & damselflies. Bloomsbury Natural History.
153. Testard, P. (1975). Note sur l'émergence, le sex-ratio et l'activité des adultes de *Mesogomphus genei* Selys, dans le sud de l'Espagne (Anisoptera: Gomphidae) [Note on the emergence, sex ratio and activity of adults of *Mesogomphus genei* Selys in southern Spain (Anisoptera: Gomphidae)]. Odonatologica, 4, 11-26.
154. Thompson, D. J. (1987). Regulation of damselfly populations: the effects of weed density on larval mortality due to predation. Freshwater Biology. 17, 367-371. https://doi.org/10.1111/j.1365-2427.1987.tb01056.x
155. Torralba-Burrial, A. F., Ocharan, F. J., Outomuro, D., Azpilicueta Amorín, M., & Cordero-Rivera, A. (2012). *Ophiogomhus cecilia*. Bases ecológicas preliminares para la conservación de las especies de interés comunitario en España [*Ophiogomhus cecilia*. Preliminary ecological bases for the conservation of species of community interest in Spain]. Invertebrados, 1, 50.
156. Torralba-Burrial, A., & Ocharan, F. J. (2006). Dispersión y proporción sexual en la emergencia en una población de *Sympecma fusca* (Odonata: Lestidae) en Huesca (NE de España) [Dispersion and sexual proportion in the emergency in a population of *Sympecma fusca* (Odonata: Lestidae) in Huesca (NE of Spain)]. Boletín de la Real Sociedad española de Historia Natural (Sección Biología), 101, 29-36.
157. Trottier, R. (1966). The emergence and sex ratio of *Anax junius* Drury (Odonata: Aeshnidae) in Canada. The Canadian Entomologist, 98, 794-798. https://doi.org/10.4039/Ent98794-8
158. Trottier, R. (1971). Effect of temperature on the life-cycle of *Anax junius* (Odonata: Aeshnidae) in Canada. The Canadian Entomologist, 103, 1671-1683. https://doi.org/10.4039/Ent1031671-12
159. Ubukata, H. (1974). Relative abundance and phenology of adult dragonflies at a dystrophic pond in Usubetsu, near Sapporo. Journal of the Faculty of Science, Hokkaido University Series VI. Zoology, 19, 758-776.
160. Ubukata, H. (1981). Survivorship curve & annual fluctuation in the size of emerging population of *Cordulia aenea amurensis* Selys (Odonata: Corduliidae). Japanese Journal of Ecology, 31, 335-346. https://doi.org/10.18960/seitai.31.4_335
161. Utzeri, C., & Ercoli, C. (2004). Disturbance by unpaired males prolongs postcopulatory guarding duration in the damselfly *Lestes virens* (Charpentier) (Zygoptera: Lestidae). Odonatologica, 33, 291-301.
162. Utzeri, C., & Raffi, R. (1983). Observations on the behaviour of *Aeshna affinis* (Vander Linden) at a dried-up pond (Anisoptera: Aeshnidae). Odonatologica, 12, 141-151.
163. Utzeri, C., Carchini, G., & Falchetti, E. (1988). Aspects of demography in *Lestes barbarus* (Fabr.) and *L. virens vestalis* Ramb (Zygoptera: Lestidae). Odonatologica, 17, 107-114.
164. Utzeri, C., Falchetti, E., & Carchini, G. (1983). The reproductive behaviour in *Coenagrion lindeni* (Selys) in central Italy (Zygoptera: Coenagrionidae). Odonatologica, 12, 259-278.
165. Van Noordwijk, M. (1978). A mark-recapture study of coexisting zygopteran populations. Odonatologica, 7, 353-374.
166. Waage, J. K. (1972). Longevity and mobility of adult *Calopteryx maculata* (Beauvois, 1805) (Zygoptera: Calopterygidae). Odonatologica, 3, 155-162.

167. Walker, E. M., & Corbet, P. S. (1958). The Odonata of Canada and Alaska: The Anisoptera (Vol. 2). Toronto, Canada: University of Toronto Press. https://doi.org/10.3138/9781442654198
168. Watanabe, M., & Taguchi, M. (1988). Community structure of coexisting *Sympetrum* species in the central Japanese paddy fields in autumn (Anisoptera: Libellulidae). Odonatologica, 17, 249-262.
169. Watanabe, M., & Taguchi, M. (2000). Behavioural protandry in the damselfly *Mnais pruinosa costalis* Selys in relation to territorial behaviour (Zygoptera: Calopterygidae). Odonatologica, 29, 307-316.
170. Watanabe, M., Matsuoka, H., & Taguchi, M. (2004). Habitat selection and population parameters of *Sympetrum infuscatum* (Selys) during sexually mature stages in a cool temperate zone of Japan (Anisoptera: Libellulidae). Odonatologica, 33, 169-179.
171. Werzinger, S., & Werzinger, J. (1993). Zweiter Zwischenbericht über Planbeobachtungen an der Grünen Keiljungfer (*Ophiogomphus cecilia*) im Bereich der Aurach, Lkr. Neustadt/Bad Winsheim, Mittelfranken [Second interim report on planned observations of the Green Maiden (*Ophiogomphus cecilia*) in the Aurach area, Lkr. Neustadt / Bad Winsheim, Middle Franconia]. Unpublished report 29.
172. Werzinger, S., & Werzinger, J. (1994). Dritter Zwischenbericht über Planbeobachtungen an der Grünen Keiljungfer (*Ophiogomphus cecilia*) im Bereich der Aurach in den Landkreisen Neustad/Bad Winsheim und Erlangen/Höchstadt, Mittelfranken [Third interim report on planned observations of the Green Maiden (*Ophiogomphus cecilia*) in the area of the Aurach in the districts Neustad / Bad Winsheim and Erlangen / Höchstadt, Middle Franconia]. Unpublished report 45.
173. Westermann, K., Westermann, S., Heitz, A., & Heitz, S. (1995). Schlüpfperiode, Schlüpfhabitat und Geschlechterverhältnis der Gemeinen Keiljungfer (*Gomphus vulgatissimus*) am südlichen Oberrhein [Hatching period, hatching habitat and sex ratio of the Common Keiljungfer (*Gomphus vulgatissimus*) on the southern Upper Rhine]. Naturschutz südl. Oberrhein, 1, 41-54.
174. Wildermuth, H. (1994). Populationsdynamik der Grossen Moosjungfer, *Leucorrhinia pectoralis* Charpentier, 1825 (Odonata, Libellulidae) [Population Dynamics of the Great Moss, *Leucorrhinia pectoralis* Charpentier, 1825 (Odonata, Libellulidae)]. Zeitschrift für Ökologie und Naturschutz, 3, 25-39.
175. Wildermuth, H., & Spinner, W. (1991). Visual cues in oviposition site selection by *Somatochlora arctica* (Zetterstedt) (Anisoptera: Corduliidae). Odonatologica, 20, 357-367.
176. William, J. L. (1900). British dragonflies (Odonata). London, United Kingdom: L.U. Gill.
177. Zettelmeyer, W. (1986). Populationsökologische Untersuchungen an der Kleinlibelle Lestes sponsa HANS. in einem Moorgebiet der Egge, Nordrhein-Westfalen. Telma, 16, 113-130.
178. http://animalandia.educa.madrid.org
179. http://artsdatabanken.no
180. http://carlosnotariomaroto.blogspot.mx/
181. http://collections.nmnh.si.edu/
182. http://www.treknature.com
183. http://greglasley.com/content/dragonix.html
184. http://iberianodonataucm.myspecies.info/
185. http://natuurtijdschriften.nl/
186. http://odonatacentrla.org
187. http://online-field-guide.com
188. http://tonkitisanpei-3.blog.so-net.ne.jp/
189. http://wikivisually.com/
190. http://www.americaninsects.net
191. https://www.wildscreenexchange.org/
192. http://www.biodiversidadvirtual.org/
193. http://www.biol.canterbury.ac.nz
194. http://www.bioscripts.net/

195. http://www.boldsystems.org
196. http://www.ddcr.org
197. http://www.entomology.ualberta.ca/
198. http://www.eol.org
199. http://www.geocities.jp/wxdmk355/v2/zukan/mk_tonbo.html
200. http://www.granadanatural.com/
201. http://www.habitas.org.uk/dragonflyireland
202. http://www.hampshiredragonflies.co.uk
203. http://www.libellen-drenthe.eu
204. http://www.libellennet.nl
205. http://www.museum.nantes.fr
206. http://www.odonata.dk
207. http://www.odonata.it/
208. http://www.pte.up.poznan.pl/
209. http://www.takao599museum.jp/
210. http://www5f.biglobe.ne.jp
211. http://yagopedia.com/
212. https://en.wikipedia.org
213. https://indiabiodiversity.org
214. https://ja.wikipedia.org/
215. https://sv.wikipedia.org
216. https://www.flickr.com/
217. https://www.iucn.org/
218. https://www.pugetsound.edu
219. https://www.revolvy.com
220. https://www.unine.ch/
221. odonata.jp
222. Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México, México.
223. Odonata collection, Natural History Museum, London, England.

Supplementary Material 2

Table S1. Gen-Bank access numbers for mitochondrial and nuclear genes. The species sample size is between parenthesis.

Taxon	12S (67)	Val (50)	16S (90)	COI (87)	18S (75)	5.8S (74)	28S (80)
Out-Group							
Blattella germanica	NC012901	NC012901	NC012901	NC012901	AF005243	AF005243	AF005243
Callibaetis ferrugineus	-	-	HM003973	JQ663249	AF370791	AY859557	AY859557
Oxya chinensis	NC010219	NC010219	NC010219	NC010219	AY037173	AF385193	AF416876
Sitophilus zeamais	NC030764	NC030764	NC030764	NC030764	AJ850021	AF276518	AY131071

In-Group

Anisoptera

Aeshnidae							
Aeshna affinis	EU477647	EU477647	EU477647	KJ873232	-	-	FJ596629
Aeshna cyanea	-	-	AF268609	KU180320	FN356031	KU180369	AF461203
Aeshna grandis	EU477645	EU477645	EU477645	KJ873213	-	KU180363	FJ596630
Aeshna isoceles	EU477649	EU477649	EU477649	KM096997	FN356032	FN356032	FJ596628
Aeshna juncea	EU477646	EU477646	AB707638	KR142917	AF461231	AB711415	EU424324
Aeshna mixta	EU477648	EU477648	EU477648	KF257096	-	AB706697	FJ712315
Anaciaeschna martini	-	-	AB707653	AB708597	AB706702	AB706702	AB706702
Anax imperator	EU477652	EU477652	EU477652	KU565916	FN356035	FN356035	FJ596632
Anax junius	AY749694	-	AY555559	KR143134	AY749908	-	FJ009959
Anax parthenope	EU477651	EU477651	AB707662	KC135891	AB706711	AB706711	-
Boyeria irene	EU477640	EU477640	EU477640	-	FN356042	FN356042	FJ596627
Cordulegastridae							
Cordulegaster boltonii	EU477688	EU477688	EU477688	KF584934	FN356072	FN356072	FJ596634
Corduliidae							
Cordulia aenea	EU477707	EU477707	LC017851	LC017856	FN356074	FN356074	EU424326
Epitheca bimaculata	EU477708	EU477708	EU477708	AB708897	FN356087	FN356087	FJ596564
Somatochlora alpestris	EU477700	EU477700	EU477700	AB708912	FN356163	FN356163	FJ596568
Somatochlora arctica	-	-	AB707969	AB708913	FN356164	FN356164	AB706870
Tetragoneuria cynosura	EU477709	EU477709	EU477709	KM536481	KT324334	-	FJ596566
Gomphidae							
Asiagomphus melaenops	EU477664	-	AB707719	AB708666	AB706768	AB706769	FJ596611
Asiagomphus pryeri	-	-	AB707725	AB708671	AB706774	AB706774	AB706776
Gomphus exilis	EU477656	EU477656	EU477656	KM531846	DQ008187	-	FJ596614.1
Gomphus flavipes	KX890620	-	KX890777	KX891019	-	-	-
Gomphus pulchellus	EU477663	-	EU477663	KX890969	-	-	KX890820
Gomphus vulgatissimus	EU477660	EU477660	EU477660	KX890971	FN356091	FN356091	FJ712320
Onychogomphus forcipatus	EU477669	EU477669	EU477669	KJ873220	-	-	EF417108
Onychogomphus uncatus	EU477671	EU477671	EU477671	KX891032	-	-	FJ712321
Onychogomphus viridicostus	-	-	AB707769	AB708716	-	AB706818	AB706818
Ophiogomphus cecilia	KX890627	-	KX890783	-	FN356139	FN356139	KX890853
Paragomphus genei	KX890631	-	KX890787	KU566310	-	-	KX890857
Sieboldius albardae	-	-	KF256850	KF257069	KC413803	AB706844	KF256923
Stylurus annulatus	-	-	AB707820	AB708765	AB706870	AB706870	FJ596610
Stylurus nagoyanus	EU477659	EU477659	EU477659	KX891020	AB706872	AB706872	FJ596610
Stylurus oculatus	-	-	AB707825	KX891021	AB706874	AB706874	-

Libellulidae

Brachythemis contaminata	-	-	EF640411	KT879898	AB707056	AB707056	AB707056
Crocothemis erythraea	-	-	EF631542	KC912244	DQ008200	-	GU196335
Deielia phaon	EF640417	EF640417	EF640417	KF257064	AB707066	AB707068	GU196343
Diplacodes trivialis	EF640421	EF640421	AB708023	JX306647	AB707073	AB707073	GU196342
Erythrodiplax umbrata	EF640426	EF640426	EF640426	-	-	-	-
Ladona depressa	EU477730	EU477730	EU477730	AF195741	DQ008204	-	FJ596598
Ladona fulva	EU477728	EU477728	EU477728	AF195745	DQ008205	-	FJ596602
Leucorrhinia dubia	EU477718	EU477718	EU477718	AB711447	AB707078	AB707078	FJ596604
Leucorrhinia pectoralis	EU477752	EU477752	EU477752	JN991193	AF461240	AF549562	FJ596605
Libellula pulchella	-	-	EF631551	KM532657	U65109	-	FJ009974
Libellula quadrimaculata	EU477729	EU477729	KF256841	KM537510	AB707091	AB707091	FJ712326
Orthetrum cancellatum	AF266097	AF266097	AF266097	-	-	-	-
Orthetrum coerulescens	DQ021445	DQ021445	DQ021445	KC912271	-	-	-
Orthetrum sabina	EF640404	EF640404	EF640404	KX670387	KJ802976	AB707198	AB860113
Pantala flavescens	EF640450	EF640450	KF256865	KF257083	EF680326+ HQ630635	AB707210	KF256938
Plathemis lydia	-	-	EF631552	KR146762	-	-	EF631234
Sympetrum danae	EU477740	EU477740	EU477740	KM532923	AF461243	AB707226	EU424330
Sympetrum depressiusculum	JQ772601	JQ772601	JQ772601	AB709125	JQ772551	JQ772551	AB707231
Sympetrum infuscatum	EF640442	EF640442	EF640442	KF257078	-	JQ772557	KF256932
Sympetrum obtrusum	EF640443	EF640443	EF640443	KM536697	EF636429	EF636429	-
Sympetrum pedemontanum	EF640444	EF640444	EF640444	KF257095	FN356171	FN356171	KF256950
Sympetrum sanguineum	EU477743	EU477743	EU477743	EF636237	AF461245	JQ772565	FJ596607
Sympetrum striolatum	EU477741	EU477741	EU477741	KF257086	FN356172	FN356172	FJ596608
Sympetrum vicinum	JQ772617	JQ772617	JQ772617	EF636299	JQ772568	JQ772568	-
Sympetrum vulgatum	EU477739	EU477739	EU477739	EF636247	AF461246	AB707301	FJ596606
Trithemis annulata	-	-	GU323149	KU566417	-	GU323055	-
Trithemis festiva	EF640458	EF640458	EF640458	JN817429	-	GU323051	-

Zygoptera

Calopterygidae

Calopteryx cornelia	-	-	AB707381	AB708328	Y12890	AB706430	AB127426
Calopteryx haemorrhoidalis	-	-	AF170957	-	Y12892	AJ308362	AJ308348
Calopteryx maculata	KM383926	KM383926	AF170960	JN419468	AJ459198	AJ459198	U65169
Calopteryx splendens	EU477613	EU477613	EU477613	DQ411707	Y12894	AJ308371	EF417106
Calopteryx virgo	-	-	-	DQ411709	AJ458968	AJ308359	AJ308357
Calopteryx xanthostoma	-	-	AF170954	-	AJ458971	AJ308355	-
Hetaerina americana	KM383950	KM383950	KM383983	KM383854	Y12896	KM383825	FJ009952
Hetaerina vulnerata	KM383952	KM383952	KM383989	KM383852	KM383822	KM383822	KJ579101
Mnais pruinosa	-	-	AB707408	AB708357	Y12898	AJ458985	AB127423

Coenagrionidae							
Argia moesta	-	-	JX121181	KR148130	FJ009997	-	FJ009939
Argia vivida	-	-	-	-	AY521866	-	AY125284
Cercion lindenii	EU477628	EU477628	EU477628	-	-	AJ488550	FN356089
Ceriagrion tenellum	-	-	-	KC912307	FN356055	FN356055	-
Chromagrion conditum	-	-	-	JN419474	FJ009995	KU245324	FJ009937
Coenagrion hastulatum	-	-	KM660019	KP272581	FN356063	FN356063	AF461207
Coenagrion mercuriale	-	-	KP27236	KP272538	FN356068	FN356068	KM248385
Coenagrion puella	AF232897	-	KP272335	-	AJ488546	AJ488546	KM660030
Coenagrion pulchellum	-	-	KF369678	KF369349	AJ488547	AJ488547	KF370075+ KM660042
Coenagrion scitulum	-	-	KM660001	-	FN356070	FN356070	KM660031
Enallagma cyathigerum	KF855787	-	KF855858	KC912314	FN356085	FN356085	KF855808
Erythromma najas	EU055009	-	EU055105	EF176769	-	AJ621054	EF417107+ AF461209
Ischnura cervula	AY282544	-	-	-	FN356101	FN356101	-
Ischnura elegans	GQ256005	-	KF256901	AB708506	FN356103	FN356103	KF370149
Ischnura graellsii	HQ834798	-	-	KC912318	AJ488545	AJ488545	-
Ischnura posita	AY282547	-	-	JN419852	-	-	-
Ischnura pumilio					FN356107	FN356107	
Nehalennia irene	GQ256009	-	GQ256022	KR141550	-	-	-
Pyrrhosoma nymphula	-	-	-	KU220885	FN356160	FN356160	FJ596559
Telebasis salva	EU477739	-	EU055077	-	EU055172	-	EU055270
Xanthocnemis zealandica	-	-	KM973571	KM106848	-	KU245345	KM973758
Lestidae							
Austrolestes colensonis	-	-	KM973583	EU219873	-	-	KM973769
Chalcolestes viridis	EU477617	EU477617	EU477617	HQ830311	FN356058	FN356058	EU424331
Lestes barbarus	EU477616	EU477616	EU477616	HQ830316	FN356110	FN356110	-
Lestes disjunctus	AY282554	-	EF044272	KM537699	-	-	-
Lestes dryas	EU477621	-	EU477621	KM537318	AB706407	AB706407	AB706407
Lestes rectangularis	-	-	EF044271	KM537590	-	-	FJ009953
Lestes sponsa	-	-	AB707363	AB708312	FN356114	FN356114	AF461204
Lestes virens	EU477619	EU477619	EU477619	KF369424	FN356117	FN356117	KF370159
Sympecma fusca	EU477630	EU477630	EU477630	KF369553	FN356170	FN356170	KF370312
Platycnemididae							
Platycnemis pennipes	EU477627	EU477627	EU477627	KF369498	AJ459230	AJ459230	FJ009928

Supplementary Material 3. Aditional analyses

Correlation between ASR and population sample size

Table S1. PGLS of ASR for all Odonata species as response variable and sample size as predictor variable.

All Odonata	Estimate (S.E.)	*t*	*P*
Intercept	0.556 (0.079)	7.013	**< 0.001**
Sample Size	-0.009 (0.027)	0.324	0.747

Residual S.E.$_{97}$=0.328; R^2=0.001; $F_{1,97}$=0.105; P=0.747; λ=0.242

Table S2. PGLS for Odonata species with ASR estimates by exuviae picking as response variable and sample size as predictor variable.

Exuviae	Estimate (S.E)	*t*	*P*
Intercept	0.360 (0.078)	4.64	**< 0.001**
Sample Size	0.047 (0.028)	1.723	0.090

Residual S.E. $_{60}$=0.236; R^2=0.047; $F_{1,60}$=2.970; P=0.090; λ=0.050

Table S3. PGLS for Odonata species with ASR estimates by mark-recapture method as response variable and sample size as predictor variable.

Mark-Recapture	Estimate (S.E.)	*t*	*P*
Intercept	0.706 (0.111)	6.379	**< 0.001**
Sample Size	-0.034 (0.040)	0.841	0.405

Residual S.E.$_{45}$=0.333; R^2=0.015; $F_{1,45}$=0.707; P=0.405; λ=0

Adult sex ratio differences between families

Table S4. Phylogenetic *t* test of ASR estimated by exuviae picking, between Odonata families. ASR estimates, "N" (number of species per families), value of one sample *t* test, lambda as phylogenetically signal and *P* value are show.

Family	ASR	N	*t*	λ	*P*
Aeshnidae	0.443	11	2.156	0	0.063
Cordulegastridae	0.472	1	-------	-------	-------
Corduliidae	0.473	5	0.736	0	0.538
Gomphidae	0.471	15	1.130	0	0.129
Libellulidae	0.467	16	1.413	1	0.181
Coenagrionidae	0.514	8	1.851	0	0.123
Lestidae	0.472	6	1.408	0	0.254

Table S5. Phylogenetic *t* test of ASR estimated by mark-recapture method, between Odonata families. ASR estimates, "N" (number of species per families), value of one sample *t* test, lambda as phylogenetically signal and *P* value are show.

Family	ASR	N	*t*	λ	*P*
Aeshnidae	0.701	2	3.330	0	-------
Libellulidae	0.537	11	0.447	1	0.667
Calopterygidae	0.662	9	3.235	0.58	**0.018**
Coenagrionidae	0.656	19	4.755	0	**<0.001**
Lestidae	0.625	5	7.563	0	**0.017**
Platycnemididae	0.543	1	-------	-------	-------

Models to test the variable sources linked to adult sex ratio

Table S6. Parameter estimates from saturated PGLS model explaining ASR variation among Odonata recently emerged as adults (exuviae method; N= 48 species).

	Estimate (S.E.)	*t*	Coefficient (95% CI)
Intercept	-1.842 (0.232)	7.94	-2.297 − -1.387
Mating System	-1.398 (0.263)	5.316	-1.913 − -0.883
Body Size	-0.878 (0.146)	6.015	-1.164 − -0.592
Sexual Size Dimorphism	0.016 (2.507)	0.006	-1.898 − 4.930
Male Wing Pigment Proportion	7.468 (2.690)	2.776	2.196 − 12.220
Mating System * Body Size	0.901 (0.163)	5.541	0.582 − 2.220
Mating System * Sexual Size Dimorphism	-0.003 (0.333)	0.008	-0.656 − 0650
Mating System * M. Wing Pigment Proportion	-7.068 (2.321)	3.046	-11.617 − -2.519
Body Size * Sexual Size Dimorphism	0.199 (1.450)	0.138	-2.643 − 3.041
Body Size * M. Wing Pigment Proportion	-0.288 (0.828)	0.347	-1.911 − 1.335
Sexual Size Dimorphism * M. Wing Pigment Proportion	1.434 (8.434)	0.17	-15.097 − 17.965

Table S7. Best PGLS model (Δ AICc < 2 and lowest number of parameters) predicting ASR variation in Odonata at emergence as adults (exuviae method) with MS, BS, SSD, and WP as predictor variables.

Model components	*k*	Δ AICc	w_i	λ	R^2 (%)
MS + BS + SSD + WP + (MS*BS) + (MS*MP)	7	0	0.309	0	0.577
MS + BS + SSD + WP + (MS+BS) + (MS*WP) + (BS*WP)	8	1.387	0.154	0	0.59
MS + BS + SSD + WP + (MS*BS) + (MS*WP) + (SSD*WP)	8	1.895	0.12	0	0.586

MS= mating system, BS= body size, SSD= sexual size dimorphism, WP= male wing pigment proportion. k= number of parameters in the model. Δ AICc= differences between AICc models. w_i= Akaike weight. λ = phylogenetically signal. R^2(%)= measure of model fit.

Table S8. Parameter estimates from saturated PGLS model explaining ASR variation among Odonata sexually mature (mark-recapture method; N=45 species).

	Estimate (S.E.)	*t*	Coefficient (95% CI)
Intercept	0.773 (0.516)	1.496	-0.238 − 1.784
Mating System	-0.333 (0.441)	0.076	-1.197 − 0.531
Body Size	-0.093 (0.342)	0.272	-0.763 − 0.577
Sexual Size Dimorphism	-1.883 (13.61)	0.138	-28.562 − 24.796
Male Wing Pigment Proportion	-0.343(0.748)	0.458	-1.809 − 1.123
Mating System * Body Size	0.036 (0.283)	0.126	-0.518 − 0.591
Mating System * Sexuel Size Dimorphism	1.878 (2.039)	0.921	-2.118 − 5.874
Mating System * M. Wing Pigment Proportion	0.183 (0.440)	0.415	-0.679 − 1.0454
Body Size * Sexual Size Dimorphism	0.717(90.07)	0.079	-17.060 − 18.494
Body Size * M. Wing Pigment Proportion	0.420 (0.374)	0.112	-0.313 − 1.153
Sexual Size Dimorphism * M. Wing Pigment Proportion	0.259 (2.491)	0.104	-4.623 − 5.141

Table S9. Best PGLS model (Δ AICc < 2 and lowest number of parameters) predicting ASR variation in Odonata at sexual maturity (mark-recapture method) with MS, BS, SSD, and WP as predictor variables.

Model components	k	Δ AICc	w_i	λ	R^2 (%)
MS + BS + SSD + WP + (MS*SSD)	6	0	0.175	0	0.128
MS + BS + SSD + WP + (MS*SSD) +(MS*WP)	7	1.675	0.076	0	0.15

MS= mating system, BS= body size, SSD= sexual size dimorphism, WP= male wing pigment proportion. k= number of parameters in the model. Δ AICc= differences between AICc models. w_i= Akaike weight. λ = phylogenetically signal. R^2(%)= measure of model fit.

Patterns of sexual dimorphism in flight agility in territorial and non-territorial Odonata

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SHORT COMMUNICATION

Patterns of sexual dimorphism in flight agility in territorial and non-territorial Odonata

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Abstract

Adult dragonflies (Anisoptera) and damselflies (Zygoptera) are amongst the most accomplished flying insects on the planet. The main functions of spatial displacement by flight in these insects are well understood (e.g., escape from predators, foraging, reproduction, thermoregulation), but whether males and females exhibit different degrees of flight agility across species—and why—is by contrast, poorly understood. This is important because flight agility may differ between males and females due to the costs imposed on females by the high levels of sexual selection and sexual conflict observed in certain species. Here we used a wing parameter to estimate sexual dimorphism in flight agility in 63 Odonata taxa. We then used a phylogenetic comparative method to investigate whether sexual dimorphism in flight agility differed between (i) Anisoptera and Zygoptera, and (ii) mating systems (non-territorial, territorial). Our results first show that the distribution of sexual dimorphism in flight agility between Odonata families is non-random. Second, our results suggest that whereas sexual dimorphism in flight agility is not different between non-territorial and territorial Anisoptera, in Zygoptera it is predominantly female-biased in non-territorial species, and male-biased in territorial ones. There may be important behavioural and mating differences between Anisoptera and Zygoptera which explain the different needs of agility observed between suborders and mating systems.

Keywords Flight agility · Mating system · Odonata · Sexual dimorphism

Introduction

In many insect species, flight is directly related to spatial displacement, feeding, survival and reproduction (Marden 2000). Dragon- and damselflies (Insecta: Odonata) are amongst the best examples illustrating selection for improved flight performance due to natural and sexual selection (Dudley 2002). The males of virtually all odonate species locate feeding and breeding grounds, capture prey in the air, and evade predators via flight. Also, the males of many species attempt to capture females during chases, which is likely to select for increased manoeuvrability in the context of sexual selection (Thornhill and Alcock 1983; Dudley 2002). The different mating behaviour of the males has been used to classify species as either territorial (resource defence polygyny) or non-territorial (non-resource defence polygyny) (Conrad and Pritchard 1992). In territorial species, one of the key determinants of male mating success is the acquisition of a territory, as females are typically attracted to these for copulations (Suhonen et al. 2008).

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In these species, males actively fight each other over the possession of a territory. In non-territorial species, males do not establish territories, and instead wander along water bodies actively looking for females (e.g., Fincke 1985). In such species, males typically exhibit scramble competition polygyny, which is favoured over territory or female-defence when neither females nor the resources used by these are economically defendable (Thornhill and Alcock 1983; Dickinson 1992). The males search or patrol looking for females, and male–male competition is indirect, because the success in acquiring mates depends on the ability of males to locate these, or on encounter rate (Dickinson 1992). Female choice is absent in this mating system; thus, male traits (e.g., flight display or body colouration) evolve under other selective pressures (e.g., to reduce intrasexual harassment; Khan and Herberstein 2019). Since particular selective pressures operate on the individuals of species belonging to either mating system, important morphological and behavioural differences between territorial and non-territorial species are likely to evolve (Conrad and Pritchard 1992).

In territorial species, for instance, male wing pigmentation is a more prevalent trait (Carrillo-Muñoz et al. in preparation), and the patterns of sexual size dimorphism (SSD) change according to mating system and male flight agility (Serrano-Meneses et al. 2008). Also, in territorial species, low flight agility males tend be larger than females, whilst high flight agility males are usually similar sized to (or even smaller than) females (Serrano-Meneses et al. 2008). SSD, by contrast, is not related to male flight agility in non-territorial species. How can these results be explained? A potential explanation to these patterns lies in the behaviour of males, and the energetics of flight. Male-biased SSD was generally expected in territorial species (e.g., Sokolovska et al. 2000; Johansson et al. 2005) given the mating advantages of large body size. Nonetheless, large body size is not always selectively advantageous for males, hence there are several territorial species in which males are smaller than females (e.g., *Plathemis lydia* and *Sympetrum rubicundulum*; Koenig and Albano 1987; Van Buskirk 1987; respectively). The males of many territorial species commonly defend their territories from other males whilst on the wing, hardly ever perching. In such species, large body size could become a disadvantage, since small body size could have lower flying costs per unit time, by contrast. Small males would then be able to allocate more time to sexual activity (i.e., searching for females), rather than foraging ('Ghiselin-Reiss, small-male hypothesis', Blanckenhorn et al. 1995). As a consequence, small male body size may become increasingly advantageous, and monomorphism or female-biased SSD would evolve. Such males may not only be better at defending territories, but also at securing copulations. This is likely, given that small male size may relate inversely to the amount of flight muscle in certain species, which would result in lower power output per unit body size, negatively affecting maximum acceleration and manoeuvrability. For instance, in the territorial dragonfly *Libellula quadrimaculata*, non-territorial males are larger and less successful (in terms of mating) than territorial males (Convey 1989).

Male morphology in Odonata may thus change to maximise male fitness (e.g., to reduce male–male mating attempts; Sherratt and Forbes 2001; Khan and Herberstein 2019), and here we are particularly interested in changes that maximise mating success. Males are known to harass females in several odonate species (e.g., Samejima and Tsubaki 2010; Takahashi and Watanabe 2010), therefore it would be in the female's best interest to minimise the negative effects of male harassment (Parker 1979). There may be several ways in which females reduce harassment from males (see Schultz and Fincke 2013), and the ability to outmanoeuvre and escape from chasing males may be one of them. For instance, in the territorial damselfly *Calopteryx splendens*, males actively chase females at high male densities, but they fail to catch up with them (Hilfert-Rüppell and Rüppell 2009). Although Hilfert-Rüppell and Rüppell (2009) attribute this observation mostly to male behaviour (males fly erratically to signal to other males as well as to females whilst in pursuit), flight speed in Odonata typically depends on body- and wing size, wing shape, wing loading and kinematic parameters of wing beats (Rüppell 1989). Therefore, we hypothesise that male and female flight agility may differ amongst odonate taxa, in response to high levels of competition over mates.

In this study, we investigated whether flight agility was different between males and females (i) across a sample of Odonata families, and (ii) amongst territorial and non-territorial species of dragonflies (Anisoptera) and damselflies (Zygoptera). We specifically predicted the males of territorial species to exhibit higher levels of flight agility than females (compared to non-territorial species), given the higher operating levels of sexual selection.

Methods

Data

Data on mating systems (non-territorial, territorial) were obtained from published sources, and from museum specimens we obtained photographs of the fully extended wings of males and females using a digital camera (Canon 20D with a 60 mm macro lens). The photographs of the right wings of males and females were used to estimate the non-dimensional radius of the second moment of wing area ($r_2(S)$) for all 63 odonate taxa (34 Anisoptera, 29 Zygoptera) for which we were able to obtain data on mating system and DNA sequences to construct a phylogenetic tree (see below). The values of both, the anterior and posterior wings, were averaged to obtain a single $r_2(S)$ value per species. Non-dimensional moments of wing area are functions of wing shape, since they depend exclusively on the distribution of the non-dimensional chord along the wing (Betts and Wootton 1988). $r_1(S)$, the radius of the first moment of wing area, denotes the position of the wing's centroid, whilst the radius of gyration, $r_2(S)$, refers to the distribution of wing area along the wing axis, and it is proportional to the mean lift force in a quasi-steady-state aerodynamic analysis, which suggests it is important for the energetics of flight (Weis-Fogh 1973; Ellington 1984). Low values of $r_2(S)$ denote a high basal distribution of wing area, and a wider range of available flight speeds at lower energy costs (Outomuro et al. 2013). Thus, wings with extended, narrow tips could benefit from high aspect ratio whilst maintaining relatively low moments of area, and hence high agility (Betts and Wootton 1998). $r_2(S)$ has been the focus of several studies, including insects (e.g., Betts and Wootton 1988; Wakeling and Ellington 1997), birds and bats (Ellington 1984). In particular, its relationship with wing size in calopterygid damselflies has been previously investigated (Outomuro et al. 2013), and has also been used as a proxy for male flight agility in studies of sexual size dimorphism in Odonata (e.g., Serrano-Meneses et al. 2008). Thus, here we use $r_2(S)$ as a proxy for flight agility. The full list of species, data and sources are available in Supplementary Material 1. A detailed description of how to calculate $r_2(S)$ is provided in Supplementary Material 2.

We calculated sexual dimorphism in flight agility as log_{10}(male agility) – log_{10}(female agility). This is similar to the estimation of sexual size dimorphism, as it yields data which are symmetrical around zero and do not violate the assumptions of parametrical tests (Lovich and Gibbons 1992). Because low $r_2(S)$ values denote high agility, positive values of sexual dimorphism refer to female-biased dimorphism (or species in which females are more agile than males), whereas negative values denote male-biased dimorphism (species in which males are the most agile sex).

Statistical analyses

We tested whether sexual dimorphism in flight agility was equal to monomorphism (similar flight agility between males and females) in four Anisoptera (Aeshnidae, Corduliidae, Gomphidae, Libellulidae) and two Zygoptera families (Calopterygidae, Coenagrionidae). To this end, we used a series of one-sample Wilcoxon signed rank tests, and specified mu = 0. Because of very low sample sizes ($n < 3$) we were unable to apply these tests to Cordulegastridae, Lestidae and Platycnemididae.

Phylogenetic comparative analyses

To correct for the phylogenetic non-independence of species (Harvey and Pagel 1991), we built a molecular phylogenetic tree which included all 63 odonate taxa. The description of the methodology used to build the tree, as well as accession numbers and the tree topology are provided in Supplementary Material 3.

To test whether sexual dimorphism in flight agility differed between (i) mating systems (non-territorial, territorial) and (ii) suborders (Anisoptera, Zygoptera) we used the Phylogenetic Generalised Least Squares method (PGLS; Pagel 1997; Freckleton et al. 2002). PGLS is a powerful phylogenetic comparative method that uses a phylogenetic tree (or trees) to specify the structure of its variance–covariance matrix to incorporate the phylogenetic autocorrelation of the data. The method tests the maximum likelihood of the evolutionary regression between traits (Pagel 1997). We estimated the maximum likelihood of the weighting parameter λ (Freckleton et al. 2002) to improve the fit of our data to our model, and further used λ to correct for phylogenetic effects. In the analysis, sexual dimorphism in flight agility was specified as the dependent variable, and mating systems and suborders were used as independent variables. We also tested the first order interaction term mating systems*suborders, and since it was statistically significant (see Results), it was retained in the model.

The PGLS model was carried out using the R (R Core Team 2019) package "caper" (Orme et al. 2018). The Wilcoxon signed rank tests and Figs. 1, 2 were carried out using R (R Core Team 2019).

Results

In Anisoptera, sexual dimorphism in flight agility was mostly male-biased in Gomphidae (one-sample Wilcoxon signed rank tests, $V = 0$, $P = 0.008$; Fig. 1) and Libellulidae ($V = 22.5$, $P = 0.036$), whereas it was not significantly different from monomorphism in Aeshnidae and Corduliidae ($P > 0.500$ in both cases). In Zygoptera, Calopterygidae exhibits more male-biased dimorphism ($V = 28$, $P = 0.005$), whilst Coenagrionidae is generally female-biased ($V = 27$, $P = 0.034$).

The significant interaction term mating systems*suborders suggests that sexual dimorphism in flight agility differs between mating systems when suborder is considered (Table 1). Inspection of the interaction term shows that in Anisoptera, sexual dimorphism in flight agility does not differ between non-territorial and territorial species (estimate ± SE: 0.001 ± 0.001, $t_{1,32} = 0.705$, $P = 0.486$), whilst in Zygoptera, evolutionary increases in territoriality correlate with evolutionary increases in male-biased sexual

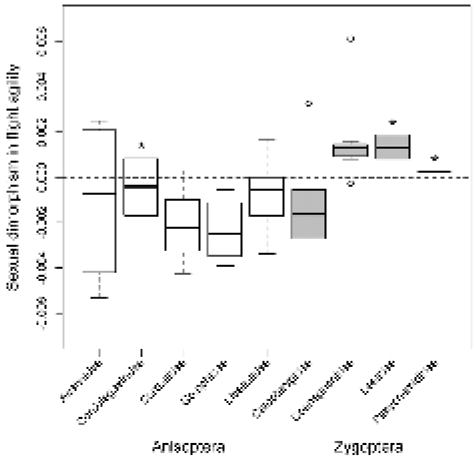

Fig. 1 Boxplot of sexual dimorphism in flight agility in nine Odonata families. The white boxes refer to Anisoptera families, whilst the grey boxes refer to Zygoptera families. Positive values of sexual dimorphism in flight agility denote female-biased dimorphism (females are more agile than males), whereas negative values denote male-biased dimorphism. The horizontal dashed line refers to monomorphism, or similar flight agility in males and females. Medians, lower- and upper quartiles, sample minimums and maximums (whiskers), as well as atypical extreme values (open circles) are shown. The asterisks show those families for which very few data were available, and tests for monomorphism were not carried out

dimorphism in flight agility (-0.002 ± 0.001, $t_{1,27} = 2.854$, $P = 0.008$; Fig. 2). Thus, in non-territorial Zygoptera, females have evolved to higher levels of flight agility than males, whereas males tend to be more agile than females in territorial species.

Discussion

Notwithstanding the exploratory nature of our study, we believe it offers valuable insights into the evolution of sexual dimorphism in flight agility in Odonata. First, we found that sexual dimorphism in flight agility differs between our sampled Odonata families. There is a range of dimorphism which spans from female-biased to male-biased flight agility. Second, we found that sexual dimorphism in flight agility can be predicted by mating system, although this is true only for Zygoptera. In Anisoptera, by contrast, sexual dimorphism in flight agility is not different between non-territorial and territorial species. Thus, our prediction that the males of territorial species would evolve to higher levels of flight agility than females is only applicable to Zygoptera.

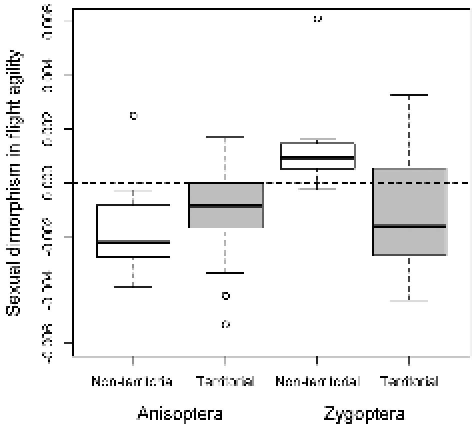

Fig. 2 Boxplot of sexual dimorphism in flight agility (dependent variable) in non-territorial (white boxes) and territorial (grey boxes) dragonflies (Anisoptera) and damselflies (Zygoptera). As in Fig. 1, positive values of sexual dimorphism denote female-biased dimorphism, and negative values denote male-biased dimorphism. The horizontal dashed line shows monomorphism. Medians, lower- and upper quartiles, sample minimums and maximums (whiskers), as well as atypical extreme values (open circles) are shown. The figure is provided for guidance only, since the data shown are species level, non-phylogenetically corrected values

Table 1 Sexual dimorphism in flight agility (dependent variable) as a function of mating system and suborders using Phylogenetic Generalised Least Squares (PGLS) in Odonata. λ is the weighting parameter in PGLS (Freckleton et al. 2002)

Independent variables	Estimate ± S.E.	*t*	*P* value
Mating systems	0.004 ± 0.002	2.084	0.042
Suborders	0.003 ± 0.001	3.099	0.003
Mating systems*Suborders	− 0.003 ± 0.001	2.633	0.011

$\lambda = 0.000$. $r^2 = 0.159$, $F_{3,39} = 3.705$, $P = 0.016$

A possible explanation to our results relates to differences between suborders in (i) wing characteristics, and (ii) the manner in which males defend their territories and search for mates. First, there are differences in aspect ratio, distribution of area over the length of the wing, and wing loading between suborders (Grabow and Rüppell 1995). Note that these are not characteristics we measured directly in our study, but these may relate to basal differences in the agility between suborders. Second, on one hand, in Anisoptera, the males of several territorial species do not perch between territorial bouts, and occasionally they even copulate on the wing. Such males spend the vast majority of time flying, patrolling their territories. The males of non-territorial species also fly for long periods, whilst they cover large areas searching for potential mates. On the other hand, the males of territorial Zygoptera tend to perch for significant periods of time between territorial bouts, and compete constantly with other males over access to females, especially at high male densities (Suhonen et al. 2008). The males of non-territorial species do not cover large areas whilst looking for mates, and spend less time or none in challenging territory owners (Suhonen et al. 2008). This may select for different needs of flight agility and flight energetics between mating systems and suborders. For instance, increased flight agility may be more relevant to males of territorial Zygoptera, given their need to catch up with females quickly, whilst trying to out-compete other males at the same time (e.g., Córdoba-Aguilar et al. 2009). By contrast, the males of non-territorial taxa engage in scramble competition, and as such, these are expected to evolve high levels of flight agility to secure copulations (e.g., Samejima and Tsubaki 2010). The females may respond to the increased selection pressure from male harassment by hiding assisted by their dull colouration and cryptic behaviour (Khan and Herberstein 2019), and/or by becoming faster and more agile, thereby matching or surpassing the levels of male flight agility. In Anisoptera, the males of non-territorial and territorial species may have similar flight agility needs, given their mating behaviour. However, we do not currently have data to make direct tests, and there is also a general lack of relevant data in the literature. For instance, to our knowledge, no study has tested energy consumption by non-territorial males in searching for mates (Suhonen et al. 2008), or thoroughly investigated the importance of flight agility to mating success. The latter may prove to be fruitful, as the females of certain dragonfly taxa are known to actively avoid male harassment, sometimes by recurring to extreme behaviours, such as dropping to the ground and stopping all movement ("drop and stop"; Wildermuth et al. 2019). The reduction of male harassment can be beneficial to females, as it can diminish the fitness costs to females from sexual conflict, thereby increasing the productivity, density and stability of a population (Takahashi et al. 2014).

Here we used a macro-evolutionary approach to investigate patterns of sexual differences in flight agility, and used a morphological wing parameter ($r_2(S)$) as a proxy for flight agility. We further used two binary variables (mating system and suborder) as predictors. We acknowledge the limitations of our approach, given that (i) the relationship between $r_2(S)$ and flight agility may not be so straightforward, and we are fully aware that a single parameter cannot properly describe flight agility (Dudley 2002), and (ii) using binary traits as predictors in PGLS models could be statistically problematic, because these contain relatively little information. Nonetheless, we believe that our study has revealed interesting, previously unknown patterns in odonates that deserve further investigation. First, an empirical test in the territorial damselfly *C. splendens* showed that females are able to escape from chasing males (Hilfert-Rüppell and Rüppell 2009). *C. splendens*, according to our calculation of sexual dimorphism in flight agility (0.0005; Supplementary Material 1), is a species in which females are expected to be more agile than males. This suggests that the use of $r_2(S)$ as a proxy for flight agility is not erroneous, as it is consistent with field observations. Second, PGLS is a robust comparative phylogenetic method which can deal with discrete data, even when used as a response variable (Jertz and Freckleton 2015).

To summarise, our results highlight patterns of sexual dimorphism in flight agility which had not been previously investigated in Odonata. Also, the current results suggest that more data is needed to explain the observed patterns. For instance, more, refined data on flight agility would prove very useful, as would do the inclusion of data on male density and sex ratios, for it would allow more precise testing beyond mating systems in Odonata. Finally, although we propose that females may respond to the evolution of high levels of male flight agility by increasing their own, it must be considered that this is not the sole form in which females avoid male harassment. In certain damselfly taxa, for example, females overcome increased male harassment by means of male mimicry, even when it can impose important costs on the fitness of males (Gering 2017).

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References

Betts CR, Wootton RJ (1988) Wing shape and flight behaviour in butterflies (Lepidoptera: papilionoidea and Hesperioidea): a preliminary analysis. J Exp Biol 138:271–288

Blanckenhorn WU, Preziosi RF, Fairbairn DJ (1995) Time and energy constraints and the evolution of sexual size dimorphism – to eat or to mate? Evol Ecol 9:369–381

Conrad KF, Pritchard G (1992) An ecological classification of odonate mating systems: the relative influence of natural, inter- and intrasexual selection on males. Biol J Linn Soc 45:255–269

Convey P (1989) Influences on the choice between territorial and satellite behaviour in male *Libellula quadrimaculata* Linn. (Odonata: Libellulidae). Behaviour 109:125–141

Córdoba-Aguilar A, Raihani G, Serrano-Meneses MA, Contreras-Garduño J (2009) The lek mating system of *Hetaerina* damselflies (Insecta: Calopterygidae). Behaviour 146:189–207

Core Team R (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Dickinson JL (1992) Scramble competition polygyny in the milkweed leaf beetle: combat, mobility, and the importance of being there. Behav Ecol 3:32–41

Dudley R (2002) Mechanisms and implications of animal flight maneuverability. Integ Comp Biol 42:135–140

Ellington CP (1984) The aerodynamics of hovering insect flight. II. Morphological parameters. Philos Trans R Soc Lond B 305:17–40

Fincke OM (1985) Alternative mate-finding tactics in a non-territorial damselfly (Odonata: Coenagrionidae). Anim Behav 33:1124–1137

Freckleton RP, Harvey PH, Pagel M (2002) Phylogenetic analysis and comparative data: a test and review of evidence. Am Nat 160:712-726

Gering EJ (2017) Male-mimicking females increase male-male interactions, and decrease male survival and condition in a female-polymorphic damselfly. Evolution 71:1390–1396

Grabow K, Rüppell G (1995) Wing loading in relation to size and flight characteristics of European Odonata. Odonatologica 24:175–186

Harvey PH, Pagel MD (1991) The comparative method in evolutionary biology. Oxford University Press, Oxford

Hilfert-Rüppell D, Rüppell G (2009) Males do not catch up with females in pursuing flight in *Calopteryx splendens* (Odonata: Calopterygidae). Int J Odonatol 12:195–203

Jertz W, Freckleton RP (2015) Towards a general framework for predicting threat status of data-deficient species from phylogenetic, spatial and environmental information. Philos Trans R Soc Lond B 370:20140016

Johansson F, Crowley PH, Brodin T (2005) Sexual size dimorphism and sex ratios in dragonflies (Odonata). Bio J Linn Soc 86:507–513

Khan MK, Herberstein M (2019) Sexually dimorphic blue bands are intrasexual aposematic signals in nonterritorial damselflies. Anim Behav 156:21–29

Koenig WD, Albano SS (1987) Lifetime reproductive success, selection, and the opportunity for selection in the white-tailed skimmer *Plathemis lydia* (Odonata: Libellulidae). Evolution 41:22–36

Lovich JE, Gibbons JW (1992) A review of techniques for quantifying sexual size dimorphism. Growth Dev Aging 56:269–281

Marden JH (2000) Variability in the size, composition, and function of insect flight muscles. Annu Rev Physiol 62:157–178

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W (2018) caper: comparative analyses of phylogenetics and evolution in R. R package version 1.0.1. https://CRAN.R-project.org/package=caper

Outomuro D, Adams CD, Johansson F (2013) Wing shape allometry and aerodynamics in calopterygid damselflies: a comparative approach. BMC Evol Biol 13:118

Pagel M (1997) Inferring evolutionary processes from phylogenies. Zool Scr 26:331–348

Parker GA (1979) Sexual selection and sexual conflict. In: Blum MS, Blum NA (eds) Sexual selection and reproductive competition in insects. Academic, New York, pp 123–166

Rüppell G (1989) Kinematic analysis of symmetrical flight manoeuvres of Odonata. J Exp Biol 144:13–42

Samejima Y, Tsubaki Y (2010) Body temperature and body size affect flight performance in a damselfly. Behav Ecol Sociobiol 64:685–692

Schultz TD, Fincke OM (2013) Lost in the crowd or hidden in the grass: signal apparency of female polymorphic damselflies in alternative habitats. Anim Behav 86:923–931

Serrano-Meneses MA, Córdoba-Aguilar A, Azpilicueta-Amorín M, González-Soriano E, Székely T (2008) Sexual selection, sexual size dimorphism and Rensch's rule in Odonata. J Evol Biol 21:1259–1273

Sherratt TM, Forbes MR (2001) Sexual differences in coloration of Coenagrionid damselflies (Odonata): a case of intraspecific aposematism? Anim Behav 62:653–660

Sokolovska N, Rowe L, Johansson F (2000) Fitness and body size in mature odonates. Ecol Entomol 25:239–248

Suhonen J, Rantala MJ, Honkavaara J (2008) Territoriality in odonates. In: Córdoba-Aguilar A (ed) Dragonflies and damselflies: model organisms for ecological and evolutionary research. Oxford University Press, Oxford, pp 203–217

Takahashi Y, Watanabe M (2010) Female reproductive success is affected by selective male harassment in the damselfly *Ischnura senegalensis*. Anim Behav 79:211–216

Takahashi Y, Kagawa K, Svensson EI, Kawata M (2014) Evolution of increased phenotypic diversity enhances population performance by reducing sexual harassment in damselflies. Nat Commun 5:4468

Thornhill R, Alcock J (1983) The evolution of insect mating systems. Harvard University Press, Cambridge

Van Buskirk J (1987) Influence of size and date of emergence on male survival and mating success in a dragonfly, *Sympetrum rubicundulum*. Am Midl Nat 118:169–176

Wakeling JM, Ellington CP (1997) Dragonfly flight III. Lift and power requirements. J Exp Biol 200:583–600

Weis-Fogh T (1973) Quick estimates of flight fitness in hovering animals, including novel mechanisms for lift production. J Exp Biol 59:169–230

Wildermuth H, Jödicke R, Schröter A, Borkenstein A (2019) Death feigning in sexual conflict between dragonflies (Odonata): does it exist? Odonatologica 48:211–228

Supplementary Material 1

Table S1. Data used in the study and sources. T = territorial; NT = non-territorial. Sample size refers the number of individuals used to calculate $r_2(S)$ per sex; we used the same number of males and females per species, according to the availability of museum specimens. Given that low values of $r_2(S)$ denote individuals with high flight agility, positive values of sexual dimorphism in flight agility (SDFA) denote taxa in which females are more agile than males, whereas negative values of SDFA denote taxa in which males are the most agile sex. SDFA = (log_{10} male $r_2(S)+1$) – (log_{10} female $r_2(S)+1$). (1) Arai (1985); (2) Azpilicueta-Amorín, M., unpub. data; (3) Brooks and Lewington (2004); (4) Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico; (5) Corbet (1999); (6) Cordero-Rivera (1988); (7) Córdoba-Aguilar (2002); (8) Córdoba-Aguilar et al. (2007); (9) Dunkle (1989); (10) Dunkle (2000); (11) Fincke, O. M., pers. comm.; (12) Johansson et al. (2005); (13) Koskimäki et al. (2004); (14) Kumar and Prasad (1977); (15) Manolis (2003); (16) McVey (1988); (17) Natural History Museum, Odonata collection, London, UK; (18) Novelo-Gutiérrez, R., pers. comm.; (19) Ocharán (1987); (20) Plaistow and Siva-Jothy (1996); (21) Serrano-Meneses et al. (2007); (22) Silsby and Parr (2001); (23) Siva-Jothy et al. (1998); (24) Sokolovska et al. (2000); (25) Tynkkynen et al. (2006); (26) Walker (1958); (27) Watanabe et al. (1998).

Species	Suborder	Mating System	$r_2(S)$ Male	$r_2(S)$ Female	SDFA	Sample size	Source
Aeshna grandis	Anisoptera	T	0.548	0.559	-0.0031	5	12, 17
Anaciaeschna isosceles	Anisoptera	T	0.548	0.567	-0.0053	5	3, 17
Anotogaster sieboldii	Anisoptera	T	0.548	0.545	0.0008	5	1, 17
Archineura incarnata	Zygoptera	T	0.565	0.574	-0.0025	5	5, 17
Atrocalopteryx atrata	Zygoptera	T	0.600	0.611	-0.0030	5	5, 17
Boyeria irene	Anisoptera	NT	0.568	0.559	0.0025	5	17, 19
Brachytron pratense	Anisoptera	T	0.566	0.560	0.0017	5	3, 17, 19
Calopteryx aequabilis	Zygoptera	T	0.597	0.603	-0.0016	5	15, 17
Calopteryx amata	Zygoptera	T	0.606	0.594	0.0033	5	5, 17
Calopteryx cornelia	Zygoptera	T	0.587	0.584	0.0008	5	5, 17
Calopteryx haemorrhoidalis	Zygoptera	T	0.594	0.596	-0.0005	5	7, 17
Calopteryx japonica	Zygoptera	T	0.594	0.600	-0.0016	5	17, 27
Calopteryx maculata	Zygoptera	T	0.599	0.609	-0.0027	5	17, 24
Calopteryx splendens	Zygoptera	T	0.593	0.591	0.0005	5	17, 25
Calopteryx virgo	Zygoptera	T	0.595	0.602	-0.0019	5	13, 17
Calopteryx xanthostoma	Zygoptera	T	0.595	0.601	-0.0016	5	17, 20
Celithemis eponina	Anisoptera	NT	0.546	0.547	-0.0003	5	5, 17
Cordulegaster boltonii	Anisoptera	T	0.548	0.554	-0.0017	5	5, 17, 19
Crocothemis erythraea	Anisoptera	T	0.548	0.554	-0.0017	5	2, 3
Dromogomphus spinosus	Anisoptera	NT	0.552	0.555	-0.0008	4	17, 26
Echo modesta	Zygoptera	T	0.598	0.608	-0.0027	3	5, 17
Enallagma cyathigerum	Zygoptera	NT	0.622	0.618	0.0011	3	4, 15

Erythemis simplicicollis	Anisoptera	T	0.544	0.550	-0.0017	5	4, 16
Gomphus exilis	Anisoptera	NT	0.555	0.568	-0.0036	5	17, 26
Gomphus externus	Anisoptera	NT	0.549	0.563	-0.0039	5	15, 17
Gomphus graslini	Anisoptera	NT	0.555	0.563	-0.0022	5	15, 17
Hetaerina americana	Zygoptera	T	0.576	0.592	-0.0044	10	21
Hetaerina titia	Zygoptera	T	0.584	0.598	-0.0038	5	8, 17
Ischnura cervula	Zygoptera	NT	0.640	0.641	-0.0003	5	4, 15, 17
Ischnura perparva	Zygoptera	NT	0.637	0.632	0.0013	5	4, 15, 17
Ischnura ramburii	Zygoptera	NT	0.645	0.622	0.0061	5	4, 9
Ladona depressa	Anisoptera	T	0.544	0.544	0.0000	5	5, 17
Ladona fulva	Anisoptera	T	0.555	0.556	-0.0003	5	3, 17
Lestes disjunctus	Zygoptera	NT	0.630	0.627	0.0008	5	17, 24
Lestes viridis	Zygoptera	T	0.625	0.618	0.0019	5	2, 6
Libellula incesta	Anisoptera	T	0.554	0.548	0.0017	5	10, 17
Libellula luctuosa	Anisoptera	T	0.536	0.548	-0.0034	3	4, 24
Libellula needhami	Anisoptera	T	0.555	0.550	0.0014	5	4, 10, 17
Libellula pulchella	Anisoptera	T	0.549	0.551	-0.0006	5	15, 17
Libellula quadrimaculata	Anisoptera	T	0.552	0.550	0.0006	5	2, 12
Macromia amphigena	Anisoptera	T	0.535	0.550	-0.0042	5	5, 17
Macromia splendens	Anisoptera	T	0.545	0.544	0.0003	5	2
Matrona basilaris	Zygoptera	T	0.602	0.598	0.0011	5	17, 23
Megaloprepus caerulatus	Zygoptera	T	0.637	0.632	0.0013	5	5, 11, 17
Mnais pruinosa	Zygoptera	T	0.602	0.613	-0.0030	5	5, 17
Neurobasis chinensis	Zygoptera	T	0.585	0.587	-0.0005	5	14, 17
Onychogomphus uncatus	Anisoptera	T	0.556	0.558	-0.0006	5	12, 17, 19
Ophiogomphus severus	Anisoptera	T	0.554	0.559	-0.0014	4	10, 17
Orthemis ferruginea	Anisoptera	T	0.542	0.547	-0.0014	4	4, 15
Pachydiplax longipennis	Anisoptera	T	0.555	0.556	-0.0003	5	4, 15, 17
Perithemis tenera	Anisoptera	T	0.558	0.558	0.0000	5	4, 15
Phaon iridipennis	Zygoptera	T	0.598	0.604	-0.0016	5	5, 17
Phyllogomphoides albrighti	Anisoptera	T	0.55	0.562	-0.0033	3	10, 17
Platycnemis pennipes	Zygoptera	NT	0.636	0.635	0.0003	5	3, 17
Pseudostigma aberrans	Zygoptera	NT	0.638	0.632	0.0016	4	17, 18
Sapho bicolor	Zygoptera	T	0.600	0.605	-0.0014	5	17, 22
Somatochlora metallica	Anisoptera	NT	0.548	0.556	-0.0022	5	3, 17
Stylurus amnicola	Anisoptera	NT	0.562	0.572	-0.0028	3	10, 17
Sympetrum corruptum	Anisoptera	T	0.538	0.541	-0.0008	5	10, 17
Sympetrum illotum	Anisoptera	T	0.540	0.549	-0.0025	5	15, 17
Telebasis salva	Zygoptera	NT	0.632	0.629	0.0008	5	15, 17
Tramea lacerata	Anisoptera	NT	0.528	0.534	-0.0017	5	4, 15
Tramea onusta	Anisoptera	T	0.532	0.536	-0.0011	5	10, 17

References

1. Arai Y (1985) An ecology of *Anotogaster sieboldii*. Kanagawa-Chuho 74:7-11. –[Jap.]
2. Azpilicueta-Amorín M, unpublished results.
3. Brooks S, Lewington R (2004) Field guide to the dragonflies and damselflies of Great Britain and Ireland. British Wildlife Publishing, Hampshire.
4. Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico.
5. Corbet PS (1999) Dragonflies: behaviour and ecology of Odonata. Harley Books, Martins, England.
6. Cordero-Rivera A (1988) Estudio ecológico de una población de *Lestes viridis* Vander Linden, 1825 (Zygoptera: Lestidae). Limnética 4:1-8.
7. Córdoba-Aguilar A (2002) Wing pigmentation in territorial male damselflies, *Calopteryx haemorrhoidalis*: a possible relation to sexual selection. Anim Behav 63:759-766.
8. Córdoba-Aguilar A, Lesher-Treviño AC, Anderson CN (2007) Sexual selection in *Hetaerina titia* males: a possible key species to understand the evolution of pigmentation in calopterygid damselflies (Odonata: Zygoptera). Behaviour 144:931-952.
9. Dunkle SW (1989) Dragonflies of the Florida Peninsula, Bermuda and the Bahamas. Scientific Publishers Nature, Gainesville, Florida.
10. Dunkle SW (2000) Dragonflies through binoculars. Oxford University Press, Oxford, England.
11. Fincke OM, personal communication.
12. Johansson F, Crowley PH, Brodin T (2005) Sexual size dimorphism and sex ratios in dragonflies (Odonata). Biol J Linn Soc 86:507-513.
13. Koskimäki J, Rantala MJ, Taskinen J, Tynkkynen K, Suhonen J (2004) Immunocompetence and resource holding potential in the damselfly *Calopteryx virgo* L. Behav Ecol 15:169-173.
14. Kumar A, Prasad M (1977) Reproductive behaviour in *Neurobasis chinensis chinensis* (Linnaeus) (Zygoptera: Calopterygidae). Odonatologica 6:163-171.
15. Manolis T (2003) Dragonflies and damselflies of California. University of California Press, Berkeley and Los Angeles, California.
16. McVey ME (1988) The opportunity for sexual selection in a territorial dragonfly, *Erythemis simplicicollis*. In: Clutton-Brock TH (ed) Reproductive success. University of Chicago Press, Chicago, Illinois, pp 44-58.
17. Natural History Museum, Odonata collection, London, UK.
18. Novelo-Gutiérrez R, personal communication.
19. Ocharán FJ (1987) Los Odonatos de Asturias y de España. Aspectos Sistemáticos y Faunísticos. PhD Thesis, Universidad de Oviedo, Spain.
20. Plaistow S, Siva-Jothy MT (1996) Energetic constraints and male mate-securing tactics in the damselfly *Calopteryx splendens xanthostoma* (Charpentier). Proc R Soc Lond B 263:1233-1238.
21. Serrano-Meneses MA, Córdoba-Aguilar A, Méndez V, Layen SJ, Székely T (2007) Sexual size dimorphism in the American Rubyspot: male body size predicts male competition and mating success. Anim Behav 73:987-997.
22. Silsby J, Parr MJ (2001) Dragonflies of the world. Smithsonian Institution Press, Washington, DC.
23. Siva-Jothy MT, Tsubaki Y, Hooper RE (1998) Decreased immune response as a proximate cost of copulation and oviposition in a damselfly. Physiol Entomol 23:274-277.
24. Sokolovska N, Rowe L, Johansson F (2000) Fitness and body size in mature odonates. Ecol Entomol 25:239-248.
25. Tynkkynen K, Kotiaho JS, Luojumäki M, Suhonen J (2006) Interspecific territoriality in *Calopteryx* damselflies: the role of secondary sexual characters. Anim Behav 71:299-306.
26. Walker EM (1958) The Odonata of Canada and Alaska, Vol. 2: Anisoptera. University of Toronto Press, Toronto.
27. Watanabe M, Taguchi M, Ohsawa N (1998) Population structure of the damselfly *Calopteryx japonica* Selys in an isolated small habitat in a cool temperate zone of Japan (Zygoptera: Calopterygidae). Odonatologica 27:213-224.

Supplementary Material 2

Protocol for calculating wing area, and wing shape. This protocol and the Excel file necessary to calculate $r_2(S)$ and other moments of wing area (see below), have been adapted from previous versions developed by Peng Chai, Robert Dudley, and Douglas Altshuler, who kindly allowed us to use these for our calculations. The Excel file "*Wing Analysis Calculations.xls*" can be obtained from the corresponding author, M. A. Serrano-Meneses; E-mail: martin.serrano@udlap.mx. The file was adapted following the calculations outlined by Ellington (1984).

Overview

This protocol provides guidance for calculating morphological features of animal wings for use in aerodynamic calculations. As such, it is not restricted to odonates. The starting point is a digital photograph of the wing that includes an internal reference scale such as graph paper or a ruler. Alternatively, the known length of the wing can be used to calibrate the image.

Image processing

Images are processed using the freeware package ImageJ, which is available for download at http://rsb.info.nih.gov/ij/.

1) Locate the image file containing the two right wings. Ideally, the full wings must be visible, from the insertion of the wings to the body all the way to the tips (see **Fig. S2.1**, in which the extended wings of a female *Celithemis eponina* are shown). Open it using a digital photo editing software, such as Adobe Photoshop. Change the image size, so that the wings do not exceed a total length of 500 pixels. Save the image file.

Figure S2.1. Female, *Celithemis eponina*.

2) Open the resized image file containing the two right wings using ImageJ (*File/Open* or Ctrl + O). Rotate the image until the leading edge vein is horizontal with respect to the screen (**Fig. S2.2**). Remember that the wing is oriented horizontally with the wing tip to the right of the screen. In the example provided, we will work using the **posterior wing**. Rotations can be made using *Image/Rotate/Arbitrarily…* and the angle can be checked using the "Angle tool".

Figure S2.2. Rotated image, with the leading edge vein of the posterior wing positioned horizontally with respect to the top of the screen.

3) Begin to set the scale by choosing the “Straight line” tool and then tracing a line on the graph paper, on a section with known distance (e.g. 1mm or 5 mm). Alternatively, trace a straight line over the full length of the wing, if the length in mm is known. This is the case for the example image shown in **Fig. S2.3**.

Figure S2.3. Straight line (yellow) traced from the insertion of the wing to the body, to the tip.

4) Once you have measured the distance in pixels between lines in the graph paper, or from the insertion of the wing to the body to the tip, you are ready to set the scale (*Analyze/Set Scale*).

Enter the distance in pixels between lines in the box entitled “Distance in Pixels”.

Enter the Known distance in mm for each grid of the graph paper in the box entitled “Known Distance.”

Enter ‘mm’ in the box entitled “Unit of Length”.

Click “Ok”.

5) Select the “Polygon selection” tool, and carefully trace around the outer edge of the wing to isolate it from the background (**Fig. S2.4**). [Save the image as an intermediate step (*File/Save* or Ctrl + S).

Figure S2.4. Edge of the wing selected using the "Polygon selection" tool (dashed line around the wing).

6) Set the image type to 8-bit grayscale (*Image/Type/8-bit*) (**Fig. S2.5**). Next, threshold the image to isolate the wing planform from the background (**Fig. S2.6**). Threshold (Image/Adjust/Threshold or Ctrl + Shift + T). Adjust the slider bars so that the wing is fully isolated from the background and the complete planform is visible (**Fig. S2.7**).

Figure S2.5. 8-bit grayscale image.

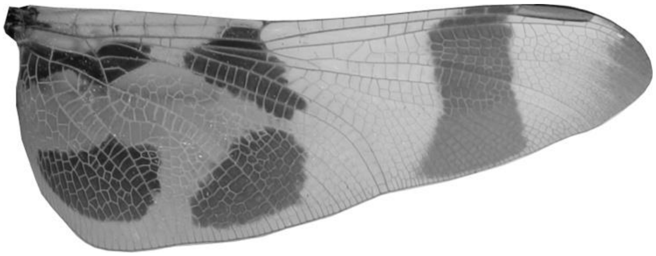


Figure S2.6. Isolated wing planform.

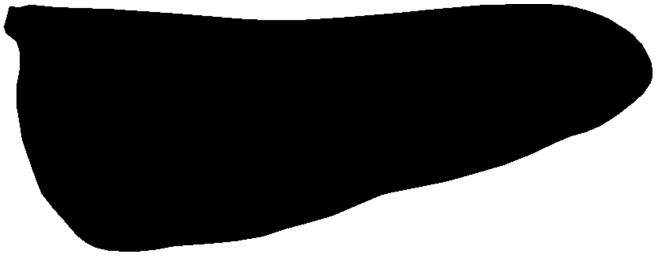


Figure S2.7. Fully isolated planform.

7) Select the wing using the “Wand (tracing) tool” and copy (*Edit/Copy* or Ctrl + C).

8) Create a new file (*File/New* or Ctrl + N) that is 500 pixels wide by 300 pixels high.

9) Paste the wing into the new file (*Edit/Paste* or Ctrl + V). [Save the image as an intermediate step (*File/Save* or Ctrl + S).

10) Apply the Lookup Table (*Image/Lookup Tables/Apply LUT*).

11) Select all (*Edit/Selection/Select All* or Ctrl + A).

12) Make a plot profile of whole image (*Analyze/Plot Profile* or Ctrl + K). **Note**: the regions where there is no wing should have “0” values, whereas the regions containing the wing should have positive values. If this pattern seems inverted, then the image must be inverted as well (*Edit/Invert* or Ctrl + Shift + I) and another plot profile must be generated.

13) Copy the plot. This can be accomplished in several ways. One of them is to hit the “Copy…” button in the plot profile window. The second is to use the menus (*Edit/Copy*) and the third is a shortcut key (Ctrl + C).

14) Open a new file in MS Excel. Paste (Ctrl + V) in the first cell of the spreadsheet. Confirm that the number of rows with entries (including zeros) is the same as the width of the ImageJ window (e.g., 500 pixels). Ignore or delete the first column. Select the cells in the second column with non-zero entries and note the number of rows. This value is the wing length in pixels. Copy the non-zero values from the second column (Ctrl + V).

15) Open the file “*Wing Analysis Calculations.xls*”. Paste into the first cell in the column entitled “profile plot”. Enter the values for height, width, wing length (mm), and wing length (pixels).

16) Save as a new file (in order to keep a copy for your records) and note the values for moments of wing area, which are provided at the bottom of the file.

References

Ellington CP (1984) The aerodynamics of hovering insect flight. II. Morphological parameters. Phil Trans R Soc Lond B 305:17-40.

Supplementary Material 3

Phylogenetic tree

In order to carry out phylogenetically corrected analyses, we constructed a molecular phylogenetic tree comprising all 63 Odonata species (34 Anisoptera, 29 Zygoptera). We included four other insect taxa as outgroup (Blattodea: *Blattella germanica*; Coleoptera: *Sitophilus zeamais*; Ephemeroptera: *Callibaetis ferrugineus*; Orthoptera: *Oxya chinensis*). Thus, we obtained the DNA sequences of four mitochondrial (12S rRNA gene: 12S; tRNA-Valine gene: Val; 16S rRNA gene: 16S; cytochrome c oxidase subunit I: COI) and three ribosomal nuclear genes (18S rRNA gene: 18S; 5.8S rRNA gene: 5.8S; 28S rRNA gene: 28S) from GenBank (NCBI Resource Coordinators 2018). These genes are commonly used in phylogenetic reconstructions of Odonata (e.g. Bybee et al. 2008; Carle et al. 2008; Dumont et al. 2010; Dijkstra et al. 2014; Letsch et al. 2016). Accession numbers for the genes used are provided in Tables S3.1 and S3.2. The sequences of each gene were independently aligned using the MUSCLE algorithm (Robert 2004) as implemented in PhyDE ver. 0.9971 (Müller et al. 2012). The aligned sequences were edited to remove any positions not present in every sequence (mitochondrial genes: 12S = 693 bp, Val = 76 bp, 16S = 1263 bp, COI = 668; nuclear genes:18S = 1969 bp, 5.8S = 163 bp, 28S = 4153 bp). The best-fit nucleotide change rate model of each gene was later estimated with jModelTest ver. 2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012) and selected according to their AICc, parameters numbers and –lnLilkelihood. The following models were selected for each gene: 12S = HKY+I+G, Val = HKY+G, 16S = HKY+I+G, COI = HKY+I+G for mitochondrial genes, and 18S = SYM+I+G, 5.8S = JC+I, 28S = GTR+I+G, for nuclear genes.

Curated alignments of the sequences of the 63 ingroup Odonata species and the four outgroup species were concatenated into a single sequence using Mesquite ver. 3.2 (Maddison and Maddison 2018). This resulted in an alignment of 8,985 bp long. This concatenated alignment and nucleotide change rate models for each gene were used to construct a posterior probability consensus tree using MrBayes ver. 3.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Altekar et al. 2004). The analysis consisted of two independent runs, each with 10,000,000 generations and four chains, sampling each 1,000 generations. The initial 25% of samples were discarded (burnin) in order to eliminate non-converging samples. The effective sampling size and parameter convergence was assessed with Tracer ver. 1.7 (Rambaut et al. 2018) for each run before mixing. Outgroup species were finally pruned from the resulting consensus tree using the R (R Core Team 2019) package *ape* (Paradis et al. 2004). Our posterior probability consensus tree (Fig. S3.1) is concordant with previous published phylogenies of Odonata for the sets of overlapping species (e.g. Bybee et al. 2008; Carle et al. 2008; Dumont et al. 2010; Dijkstra et al. 2014).

Table S3.1. GenBank accession numbers for the mitochondrial genes used to construct the phylogeny used in the study.

Outgroup	**12S**	**Val**	**16S**	**COI**
Blatella germanica	NC012901.1	NC012901.1	NC012901.1	NC012901.1
Callibaetis ferrugineus	---	---	HM003973.1	JQ663249.1
Oxya chinensis	NC010219.1	NC010219.1	NC010219.1	NC010219.1
Sitophilus zeamais	NC030764.1	NC030764.1	NC030764.1	NC030764.1
Odonata species	**12S**	**Val**	**16S**	**COI**
Aeshna grandis	EU477645.1	EU477645.1	EU477645.1	KU180299.1
Anaciaeschna isosceles	EU477649.1	EU477649.1	EU477649.1	KM096997.1
Anotogaster sieboldii	EU477686.1	EU477686.1	EU477686.1	AB708824.1
Archineura incarnata	---	---	KF369626.1	---
Atrocalopteryx atrata	---	KF256893.1	KF256893.1	KF257110.1
Boyeria irene	EU477640.1	EU477640.1	EU477640.1	---
Brachytron pratense	EU477641.1	EU477641.1	EU477641.1	KY847551.1
Calopteryx aequabilis	EU054934.1	---	KM383956.1	---
Calopteryx amata	---	---	---	DQ411628.1
Calopteryx cornelia	---	---	LC366483.1	AB708327.1
Calopteryx haemorrhoidalis	---	---	AF170957.1	---
Calopteryx japonica	---	---	AB127065.1	KF257102.1
Calopteryx maculata	KM383926.1	KM383926.1	AF170960.1	JN419468.1
Calopteryx splendens	EU477613.1	EU477612.1	AF170955.1	DQ411707.1
Calopteryx virgo	---	---	---	DQ411709.1
Calopteryx xanthostoma	---	---	AF170954.1	---
Celithemis eponina	EF640393.1	EF640393.1	AF037190.1	---
Cordulegaster boltonii	EU477688.1	EU477688.1	AF266056.1	KF584934.1
Crocothemis erythraea	AF266100.1	AF266100.1	EF631542.1	MF774557.1

Dromogomphus spinosus	EU477662.1	EU477662.1	EU477662.1	KX890955.1
Echo modesta	EU055003.1	---	KF369709.1	KF369379.1
Enallagma cyathigerum	MF716899.1	MF716899.1	MF716899.1	MF716899.1
Erythemis simplicicollis	AY282566.1	---	EF631505.1	AF195759.1
Gomphus exilis	EU477656.1	EU477656.1	AF266074.1	KM537192.1
Gomphus externus	EU477655.1	EU477655.1	KX890668.1	KX890898.1
Gomphus graslini	EU477661.1	EU477661.1	EU477661.1	---
Hetaerina americana	EU054936.1	KM383949.1	AF170951.1	MG466503.1
Hetaerina titia	KM383937.1	KM383937.1	KM383961.1	KM383863.1
Ischnura cervula	AF067705.1	---	---	---
Ischnura perparva	AF067716.1	---	GU812263.1	---
Ischnura ramburii	AF067720.1	---	---	---
Ladona depressa	EU477730.1	EU477730.1	EU477730.1	AF195741.1
Ladona fulva	EU477728.1	EU477728.1	EU477728.1	AF195745.1
Lestes disjunctus	AY282554.1	---	EF044272.1	KM537699.1
Lestes viridis	EU477617.1	EU477617.1	EU477617.1	HQ830311.1
Libellula incesta	---	---	AF037179.1	MG381974.1
Libellula luctuosa	AY282563.1	---	AF037178.1	AF195749.1
Libellula needhami	---	---	AF195730.1	AF195751.1
Libellula pulchella	---	---	EF631551.1	AF195753.1
Libellula quadrimaculata	EU477729.1	EU477729.1	EF631589.1	JN294441.1
Macromia amphigena	AF266049.1	---	AF266046.1	KF257059.1
Macromia splendens	EU477696.1	EU477696.1	EU477696.1	---
Matrona basilaris	EU054990.1	---	AB707395.1	KF369432.1
Megaloprepus caerulatus	NC031823.1	NC031823.1	NC031823.1	NC031823.1
Mnais pruinosa	---	---	AB127063.1	LC366779.1
Neurobasis chinensis	---	---	KJ730206.1	MG518624.1
Onychogomphus uncatus	EU477671.1	EU477671.1	AF266054.1	KX891032.1
Ophiogomphus severus	EU477673.1	EU477673.1	EU477673.1	KX890937.1
Orthemis ferruginea	EF640402.1	EF640402.1	EF640402.1	---
Pachydiplax longipennis	EF640433.1	EF640433.1	EF631512.1	AF195761.1
Perithemis tenera	EF640409.1	EF640409.1	EF631529.1	---
Phaon iridipennis	---	---	EU183234.1	---
Phyllogomphoides albrighti	EU477675.1	EU477675.1	EU477675.1	---
Platycnemis pennipes	EU477627.1	EU477627.1	EU477627.1	KF369498.1
Pseudostigma aberrans	JQ966649.1	---	DQ642984.1	---
Sapho bicolor	---	---	KF369896.1	KF369539.1
Somatochlora metallica	EU477702.1	EU477702.1	AB707987.1	AB708932.1
Stylurus amnicola	EU477657.1	EU477657.1	KX890710.1	KX890946.1
Sympetrum corruptum	EF640439.1	EF640439.1	EU055041.1	KM529511.1
Sympetrum illotum	EF640441.1	EF640441.1	EF640441.1	---

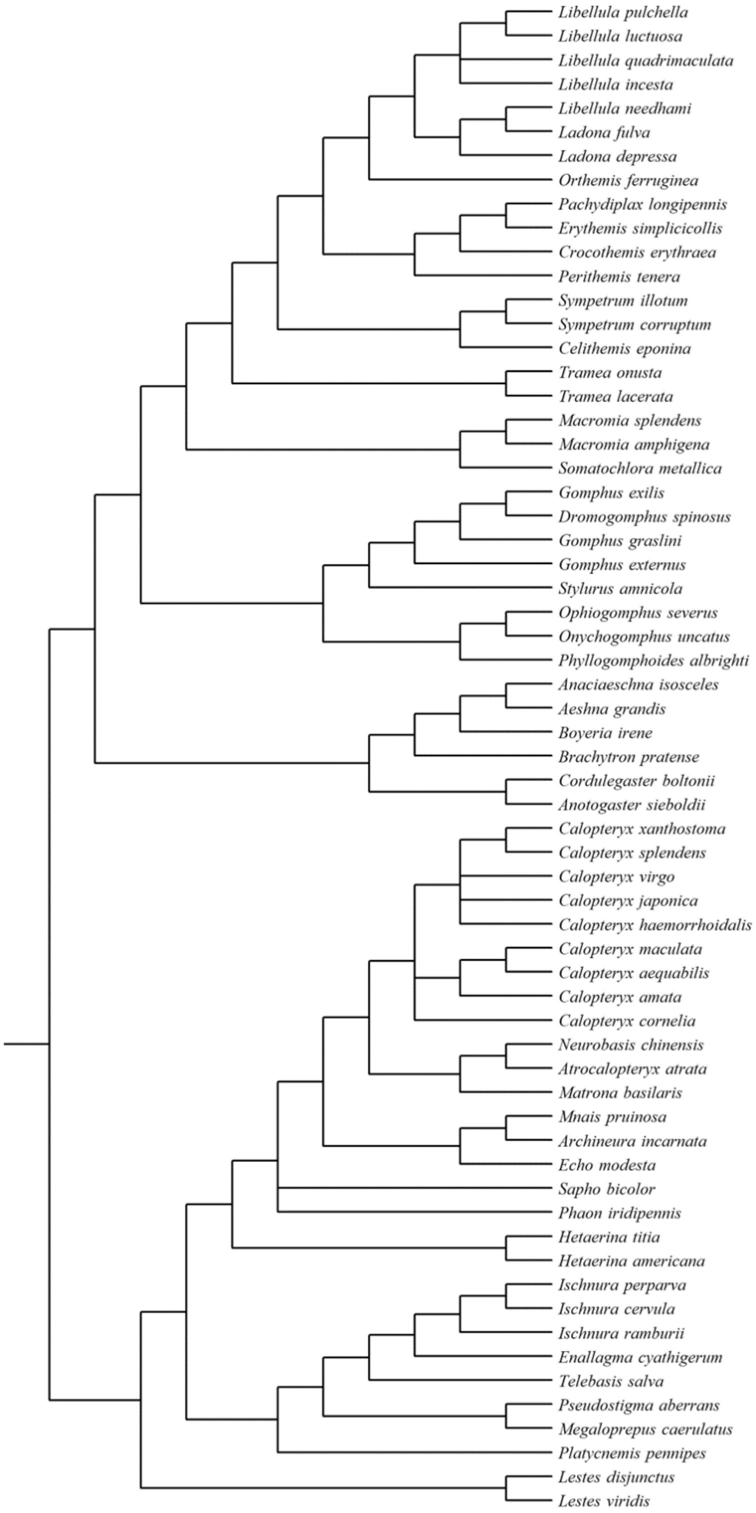
Telebasis salva	AF067721.1	---	EU055077.1	---
Tramea lacerata	EU054980.1	---	EF631538.1	AB709202.1
Tramea onusta	AY282561.1	---	EF631593.1	---

Table S3.2. GenBank accession numbers for the nuclear genes used to construct the phylogeny used in the study.

Outgroup	**18S**	**5.8S**	**28S**
Blatella germanica	AF005243.2	AF005243.2	AF005243.2
Callibaetis ferrugineus	AF370791.1	AY859557.1	AY859557.1
Oxya chinensis	AY037173.1	AF385193.1	AF416876.1
Sitophilus zeamais	AJ850021.1	AF276518.1	AY131071.1
Odonata species	**18S**	**5.8S**	**28S**
Aeshna grandis	---	KU180363.1	FJ596630.1
Anaciaeschna isosceles	DQ008199.1	FN356032.1	FJ596628.1
Anotogaster sieboldii	AB706943.1	AB706943.1	FJ596638.1
Archineura incarnata	AJ459202	AJ459202.1	KF370024.1
Atrocalopteryx atrata	KC413771.1	AB706426.1	FJ596560.1
Boyeria irene	FN356042.1	FN356042.1	FJ596627.1
Brachytron pratense	EU055199.1	FN356043.1	EU424323.1
Calopteryx aequabilis	AY338716.1	AJ308360.1	AY338673.1
Calopteryx amata	AJ458977.1	AJ308361.1	FJ009949.1
Calopteryx cornelia	Y12890.1	AB706430.1	AB127426.1
Calopteryx haemorrhoidalis	AJ458976.1	AJ308362.1	AJ308348.1
Calopteryx japonica	KC413781.1	AJ459193.1	KF256957.1
Calopteryx maculata	AJ459198.1	AJ459198.1	U65169.1
Calopteryx splendens	Y12894.1	AJ308371.1	EF417106.1
Calopteryx virgo	AJ458968.1	AJ308359.1	AJ308357.1
Calopteryx xanthostoma	AJ458971.1	AJ308355.1	AJ308355.1
Celithemis eponina	AF461233.1	AF549603.1	AF461218.1
Cordulegaster boltonii	FN356072.1	FN356072.1	FJ596634.1
Crocothemis erythraea	DQ008200.1	---	GU196335.1
Dromogomphus spinosus	DQ008189.1	---	FJ596613.1
Echo modesta	EU055194.1	AJ458984.1	EU055292.1
Enallagma cyathigerum	FN356085.1	FN356085.1	KF855808.1
Erythemis simplicicollis	---	---	EF631389.1
Gomphus exilis	DQ008187.1	---	FJ596614.1
Gomphus externus	DQ008184.1	---	FJ596612.1
Gomphus graslini	---	---	---
Hetaerina americana	Y12896.1	KM383825.1	FJ009952.1
Hetaerina titia	---	AJ458990.1	AJ458990.1

Ischnura cervula	FN356101.1	FN356101.1	FN356101.1
Ischnura perparva	FN356106.1	FN356106.1	FN356106.1
Ischnura ramburii	FN356108.1	FN356108.1	FN356108.1
Ladona depressa	DQ008204.1	---	FJ596598.1
Ladona fulva	DQ008205.1	---	FJ596602.1
Lestes disjunctus	---	---	---
Lestes viridis	FN356058.1	FN356058.1	EU424331.1
Libellula incesta	---	---	---
Libellula luctuosa	---	---	EF631392.1
Libellula needhami	---	---	---
Libellula pulchella	U65109.1	---	FJ009974.1
Libellula quadrimaculata	AB707091.1	AB707091.1	FJ712326.1
Macromia amphigena	FN356127.1	FN356127.1	FJ596570.1
Macromia splendens	DQ008195.1	FN356128.1	FJ712329.1
Matrona basilaris	AJ459206.1	AJ459206.1	EU055279.1
Megaloprepus caerulatus	FJ010033.1	---	FJ009977.1
Mnais pruinosa	Y12898.1	AJ458985.1	AB127423.1
Neurobasis chinensis	AJ459199.1	AJ459199.1	AJ459199.1
Onychogomphus uncatus	---	---	FJ712321.1
Ophiogomphus severus	DQ008192.1	---	FJ596618.1
Orthemis ferruginea	---	---	EF631266.1
Pachydiplax longipennis	---	---	EF631198.1
Perithemis tenera	FJ010032.1	---	FJ009976.1
Phaon iridipennis	AJ459225.1	AJ459225.1	AJ459225.1
Phyllogomphoides albrighti	---	---	---
Platycnemis pennipes	AJ459230.1	AJ459230.1	FJ009928.1
Pseudostigma aberrans	FJ009992.1	---	FJ009934.1
Sapho bicolor	AJ459222.1	AJ459222.1	KF370295.1
Somatochlora metallica	AB707037.1	AB707037.1	FJ596569.1
Stylurus amnicola	DQ008186.1	---	---
Sympetrum corruptum	EU055135.1	JQ772548.1	EU055230.1
Sympetrum illotum	JQ772556.1	JQ772556.1	JQ772556.1
Telebasis salva	EU055172.1	---	EU055270.1
Tramea lacerata	DQ008206.1	AB707308.1	EU055269.1
Tramea onusta	---	---	EF631281.1

Figure S3.1. Topology of the posterior probability consensus tree of Odonata used to correct the phylogenetic non-independence of data. The figure was produced using the R package *ggtree* (Yu et al. 2017).



References

Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F (2004) Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20:407-415.

Bybee SM, Ogden TH, Branham MA, Whiting MF (2008) Molecules, morphology and fossils: a comprehensive approach to odonate phylogeny and the evolution of the odonate wing. Cladistics 24:477-514.

Carle FL, Kjer KM, May ML (2008) Evolution of Odonata, with special reference to Coenagrionoidea (Zygoptera). Arthropod Syst Phylo 66:37-44.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9: 772.

Dijkstra K-DB, Kalkman VJ, Dow RA, Stokvis FR, van Tol J (2014) Redefining the damselfly families: a comprehensive molecular phylogeny of Zygoptera (Odonata). Syst Entomol 39:68-96.

Dumont HJ, Vierstraete A, Vanfleteren JR (2010) A molecular phylogeny of the Odonata (Insecta). Syst Entomol 35:6-18.

Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst Biol 52:696-704.

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755.

Letsch H, Gottsberger B, Ware JL (2016) Not going with the flow: a comprehensive time-calibrated phylogeny of dragonflies (Anisoptera: Odonata: Insecta) provides evidence for the role of lentic habitats on diversification. Mol Ecol 25:1340-1353.

Maddison WP, Maddison DR (2018) Mesquite: a modular system for evolutionary analysis. Version 3.51. http://www.mesquiteproject.org.

Müller J, Müller K, Nienhuis C, Quandt D (2012) PhyDe — Phylogenetic Data Editor. Programme distributed by the author: http://www.phyde.de/.

NCBI Resource Coordinators (2018) Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 46:D8–D13.

Paradis E, Claude J, Strimmer K (2004) Ape: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289-290.

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst Biol 67:901-904.

Robert CE (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792-1797.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Yu G, Smith DK, Zhu H, Guan Y, Lam TT-Y (2017) Ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol 8:28-36.

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Assortative Mating by Size in the American Rubyspot Damselfly (*Hetaerina americana*)

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Abstract

Assortative mating refers to the non-random nature of mating patterns between certain males and females. Thus, males and females may associate negative- or positively, based on different traits. Amongst these associations, assortative mating by size is one of the most common patterns found in natural populations of animals. Two main hypotheses have been proposed to account for the occurrence of assortative mating by size. First, it may be the result of mechanical, temporal, or physiological constraints. Second, it may occur in response to direct or indirect selection on mating preferences. Here we investigate whether the American rubyspot damselfly exhibits true assortative mating by size. Males of this species exhibit high levels of male-male competition, as they compete over territories, to which females are attracted for copulation. There is a documented large male body size advantage: the largest males are better able to hold their territories and thus secure more copulations. Our major results show that i) mated males are more likely to be larger than unmated males, whereas mated and unmated females tend to have similar body sizes; ii) *H. americana* exhibits true assortative mating by size; as such, this pattern is not driven by seasonal changes in the body sizes of males and females. We suggest that this mating pattern occurs in this species given the advantages of large male size, and the advantages of large female body size (i.e. higher fecundity). We believe that males may be able to evaluate a female's reproductive value and exert mate choice.

Keywords Assortative mating · body size · territoriality · damselfly

Introduction

There is a traditional saying in Mexico concerning the non-random nature of mating patterns between certain males and females: *dos, para quererse, deben parecerse* – to love

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one another, two must be alike. In biology, the correlated similarity between the members of a mated pair on a given genetic or phenotypic trait is known as assortative mating (Jiang et al. 2013). When assortative mating is detected, the pattern may be positive, or negative (also known as disassortative mating; Partridge 1983; Hooper and Miller 2008; but see Jiang et al. 2013). There are several empirical examples of assortative mating in animals, and these occur mostly as positive correlations on many different traits (reviewed by Jiang et al. 2013), including major histocompatibility complex alleles (Bonneaud et al. 2006), heterozygosity (García-Navas et al. 2009), age (Black and Owen 1995), colour (Krüger et al. 2001; Jawor et al. 2003), condition (Fauchier and Thomas 2001; Sharp and Agrawal 2009), and body size (Forbes et al. 1992; Masumoto 1999; Tárano and Herrera 2003). Amongst these relationships, assortative mating by size (i.e. by several traits related to body size) stands out as one of the most common forms of assortative mating in natural populations of animals (Crespi 1989; Jiang et al. 2013).

To explain the occurrence of assortative mating by size, two main hypotheses have been advanced (Crespi 1989; Jiang et al. 2013). First, assortative mating may be the incidental result of mechanical, temporal and physiological constraints (Jiang et al. 2013). There may be several mechanisms that contribute to these constraints, such as spatial and temporal segregation, intrasexual competition, and intersexual conflict (Crespi 1989; Arnqvist et al. 1996; reviewed by Jiang et al. 2013). Under this hypothesis, the resulting assortment may be selectively advantageous, neutral, or deleterious. Second, according to mate choice hypotheses, assortative mating may evolve as a response to direct or indirect selection on mating preferences. This is likely to occur if there is a male or female behavioural tendency to mate with a mate of certain size (Crespi 1989). Thus, male choice may cause assortative mating if males tend to choose large, fecund females (relative to other females) as mates (reviewed by Ridley 1983). Nonetheless, one of the main problems with this hypothesis is that male choice cannot be separated from male-male competition, as these are antagonistic processes whose effects may not coincide (Crespi 1989). For instance, in certain species with intense male-male competition, females may be mated regardless of their size, and mated males may be larger than unpaired males as a result of male-male competition (e.g. Lawrence 1986). By contrast, in species with female-biased sex ratios, males may be mated regardless of size, but mated females may be larger than unmated females as a result of male choice. In other species, without male-male competition, both males and females prefer to mate with larger-than-average individuals, and small individuals seem to be left with no option but to mate amongst themselves (Brown 1990).

In insects, assortative mating by size has been observed mainly in beetles (Bernstein and Bernstein 2003), bugs (McLain and Shure 1987; Fairbairn 1988), butterflies (Kemp 2008), and flies (Pavković-Lučić et al. 2009), but apart from a brief mention of the mating pattern by Serrano-Meneses et al. (2007), to our knowledge, it has never been properly investigated in dragonflies and damselflies (Insecta: Odonata). Odonates are an interesting group to investigate assortative mating by size and its possible causes mainly because of their mating behaviour and mating strategies. Although different mating systems have been reviewed by Corbet (1999), two are commonly used to describe the mating behaviour of a given species with different degrees of sexual selection: non-territoriality, and territoriality. Males of non-territorial taxa do not establish or defend areas which are used by females for oviposition. Males of territorial taxa fight over the acquisition of a territory (Corbet 1999), since it greatly increases male mating success, as females are attracted to these areas for copulation and/or oviposition (Córdoba-Aguilar and Cordero-Rivera 2005). Fights over a territory may be short (e.g. 3–5 s), or long (from 20 min to over 2 h; Serrano-Meneses et al. 2007); however, males commonly acquire their territories after prolonged encounters (reviewed by Córdoba-Aguilar and Cordero-Rivera 2005). In Calopterygidae, the acquisition of a territory is usually determined by the outcome of aerial encounters between residents and intruders (Córdoba-Aguilar and Cordero-Rivera 2005). In these encounters, males with higher energy reserves (metabolic fat), larger body sizes and/or higher levels of wing pigmentation, have a mating advantage over other males (Marden and Waage 1990; Grether 1996a, b; Plaistow and Siva-Jothy 1996; Koskimäki et al. 2004; Contreras-Garduño et al. 2006; Serrano-Meneses et al. 2007; Serrano-Meneses et al. 2008).

In this study, we investigate whether assortative mating by size occurs in a territorial damselfly, the American Rubyspot (*Hetaerina americana*) (Fabricius). In this species, males are larger than females (Serrano-Meneses et al. 2007) and bear red spots at the base of the wings, which are sexually selected traits maintained via male-male interactions (Grether 1996b). Males exhibit two main alternative mating tactics, non-territoriality, and territoriality (Serrano-Meneses et al. 2007; Raihani et al. 2008). Males compete intensely over the monopolisation of a territory since male mating success is largely determined by the ownership of a territory. Larger-than-average males are more likely to become and remain territorial, and also hold larger amounts of metabolic fat, which enable them to sustain long territorial fights (Serrano-Meneses et al. 2007). Non-territorial males have very low mating success (compared to territorial males) but are still able to secure a number of copulations. Thus, since variation in male morphology, mating behaviour and mating success exists between territorial and non-territorial males, here we investigate whether an association between male and female morphology is likely to exist amongst mated individuals.

Materials and Methods

Study Site

Fieldwork was carried out in Tehuixtla, Morelos, Mexico (18° 32′ 56” N, 99° 16′ 23” W, mean elevation 840 m.a.s.l.) from December the 17th 2003 to February the 27th 2004 (season 2003), and from November the 12th to December the 15th 2004 (season 2004). We worked along one of the shores of the Amacuzac river, in a section approximately 300 m long.

Marking, Morphometrics, Body Size and Age Estimation

Using an insect net, we caught unmarked individuals on a daily basis and marked them with an indelible black marker on the right anterior wing. We used a unique combination of three digits that was easily readable through binoculars from a distance of one to five metres. For each captured individual, we measured body length (from the head to the tip of the abdomen) and wing length (right anterior wing) using an electronic digital calliper (to the closest 0.01 mm). Both traits, body- and wing length, were used as *proxies* for body size. Also, by following the protocol of Córdoba-Aguilar (1994), we determined the approximate age of individuals based on morphological cues. Briefly, juvenile-mature males have bright, intense brown, green and red body colours as well as highly translucent wings; mature males show less brightness and intensity in body colouration, their wings are less translucent; old males are typically dark and their amber wings tend to be broken at the tips. Since old males tend to be non-territorial, we only included juvenile-mature and mature males in the analyses (see similar approaches in Serrano-Meneses et al. 2007 and Ramírez-Delgado et al. 2015). In this way, we aimed to minimise error when assigning males as either territorial or non-territorial (see below). Handling time was approximately 3 min for each individual.

Male Mating Behaviour and Mating Success

We carried out daily surveys between 11:00 and 14:00 h (Central Standard Time). This is the time when American rubyspots are most active (see Serrano-Meneses et al. 2007). During the surveys, we noted i) the identity of marked males and ii) whether they were seen defending an area or not. In the study site, territorial males established and defended territories against conspecifics and remained in the area after aggressive interactions with other males. By contrast, non-territorial males did not establish or defend territories and instead wandered along the study site; they were also constantly chased off by territorial males. Nonetheless, in order to minimise errors when categorising males, we considered a male as territorial if it was observed defending a particular area for at least two consecutive days. Once a male was defined as territorial, we placed a translucent plastic straw within the male's territory. The straw was marked with the identity of the territorial male and helped us to identify territorial males faster and more accurately. Finally, during the surveys, we noted the identities of those males and females observed in copula, or we captured and marked them if one or both individuals were not marked, and took the measurements described above. Since no male or female were observed copulating more than once, an individual was considered to be successful if they were observed in copula at least once.

Statistical Analyses

In order to minimise the effect of seasonality on body size variation, we only considered for analyses those copulating pairs observed during consecutive days, although we also tested directly for seasonal variation in body size (see below).

First, we tested whether the data were normally distributed by using Q-Q plots (Sokal and Rohlf 2012). Since the data did not depart from a normal distribution, we used two-way ANOVAs to investigate differences in body size amongst seasons and the sexes. In this analysis, either body- or wing length were used as variables, and season and sex were used as two-level factors. Similarly, we used two-way ANOVA to investigate differences in body size between those T and NT males observed in copula. In these analyses, body- and wing length were used as variables, and male status (T or NT) and season were used as two-level factors. In these analyses, season was included as a factor in order to account for observed differences in body size between seasons (see Results). We tested first order interaction terms in each analysis, and when a term was statistically significant, it was retained in the corresponding test. Where appropriate, *post-hoc* differences were investigated using Tukey's honestly significant difference tests.

Second, we tested for differences in body size between mated and unmated males and females using two-way ANOVAs. In the analyses, we used either body- or wing length as variables, and mating success (coded as 1, for mated individuals; or 0, for unmated ones), and season, as two-level factors. In these analyses, season is included to account for seasonal variation in mating success. First order interaction terms were investigated in each analysis and retained if statistically significant. Posterior differences were investigated using Tukey's honestly significant difference tests.

Third, we investigated seasonal changes in the body sizes of captured individuals using Pearson's product-moment correlations between the body sizes of either males or females, and the days when the individuals were observed in copula (henceforth *date*).

Fourth, to investigate assortative mating by size amongst males and females observed in copula, we used major axis regressions (MA). The advantages of this method over others, such as OLS regression (Model I regression) and reduced major axis regression (RMA) have been discussed elsewhere (e.g. Sokal and Rohlf 2012; Álvarez et al. 2013). MA regression assumes that both x and y variables are estimated with error and that error variance is equal in both variables, and it provides an estimate of the allometric slope between variables measured in the same units. Also, MA regression allows the statistical testing of the general null hypothesis $\beta = 0$, as well as the null hypothesis of isometry $\beta = 1$ (Harvey and Mace 1982; McArdle 1988; LaBarbera 1989; Fairbairn 1997). The method is known to perform better when both variables are transformed to a logarithmic scale (Sokal and Rohlf 2012); thus, data on male and female body sizes were log_{10} transformed prior to analyses. Furthermore, we assessed the magnitude and shape of triangularity of each MA regression, by following a procedure similar to the one described by Arnqvist et al. (1996). The procedure allows the observer to discriminate between true and apparent assortative mating. The apparent forms of assortative mating are linear relationships between male and female body size, with a high degree of heteroscedasticity (e.g. the data form triangular shapes around the regression line), whilst the true forms are distinguished by the symmetrical distribution of observations along the regression line (see Arnqvist et al. 1996). Thus, we regressed female body size (dependent variable) on male body size (independent variable), and we saved the residuals from these MA regressions. We then used Spearman's rank correlations to investigate the relationship between the absolute values of the residuals, and male body size. This index of heterocedasticity will be < 0 if large females are preferred over small ones, and > 0 if small females are preferred over large ones.

Finally, we constructed two lineal models, in which we tested whether the association between the body sizes of copulating males and females could be driven by changes in body size along the seasons. Thus, Model 1 included female body length as a function of male body length, date (i.e. consecutive days), and season. Model 2 included female wing length as a function of male wing length, date, and season. The interaction term male body size*date was tested in each model, but as these were not statistically significant, they were removed from the models. Season was included in the models to account for body size differences between seasons 2003 and 2004. For consistency, data on body size were log_{10} transformed prior to analyses.

Statistical analyses and figures were produced using R (ver. 3.5.0 "Joy in Playing"; R Core Team 2018). MA regressions were carried out using the 'smatr' package (Warton et al. 2012), which is used to calculate the allometric slope between two variables and their 95% confidence intervals (upper – lower CIs). A slope is considered different from $\beta = 1$ if the calculated CIs exclude $\beta = 1$ and the associated P value of the regression is ≤ 0.05. Also, the package implements a procedure to test whether a calculated slope differs from a given slope value ('slope.test'). The procedure is described in Warton et al. (2006).

Results

A total of 756 males and 144 females were marked in both seasons. From these, 72 males and 72 females were observed in copula. The body length of marked individuals differed between seasons and between the sexes (two-way ANOVA: season, $F_{1,897} = 157.800$, $P = 0.001$; sex, $F_{1,897} = 2115.400$, $P = 0.001$): individuals from the 2003 season were on average smaller than individuals from the 2004 season, and also, males were larger than females. The pattern was similar for wing length (season, $F_{1,897} = 84.530$, $P = 0.001$; sex, $F_{1,897} = 15.010$, $P = 0.001$), except that based on this trait, females are larger than males.

In males observed in copula, body length was similar amongst T and NT males (male status, $F_{1,60} = 0.166$, $P = 0.685$; season, $F_{1,60} = 15.010$, $P = 0.001$), albeit wing length changed differently amongst male status when season was considered (male status, $F_{1,59} = 0.010$, $P = 0.920$; season, $F_{1,59} = 9.806$, $P = 0.003$; male status*season, $F_{1,59} = 4.031$, $P = 0.049$). An inspection of the interaction term showed that the only significant difference in wing length occurs between T males, amongst seasons (Tukey's honestly significant difference test, $P = 0.004$): T males marked in the 2004 season had longer wings than males marked in the 2003 season.

Body Size and Mating Success

Male body length changes differently between mated and unmated males when season is considered (male mating success, $F_{1,752} = 4.888$, $P = 0.027$; season, $F_{1,752} = 143.233$, $P = 0.001$; male status*season, $F_{1,752} = 4.386$, $P = 0.037$; Fig. 1). Inspection of the interaction term suggested that, whilst in the 2003 season mated and unmated males had similar body lengths ($P = 0.995$), mated males from the 2004 season were larger than unmated males from the same season ($P = 0.013$), and also larger than mated and unmated males from the 2003 season ($P = 0.001$, in both cases). Mated males further exhibited longer wings than unmated ones (male mating success, $F_{1,753} = 9.961$, $P = 0.002$; season, $F_{1,753} = 62.003$, $P = 0.001$).

Interestingly, body size was not different amongst mated and unmated females (female mating success, $F_{1,141} = 2.875$, $P = 0.092$; season, $F_{1,141} = 60.903$, $P = 0.001$). This was also true for female wing length (female mating success, $F_{1,141} = 2.562$, $P = 0.112$; season, $F_{1,141} = 18.494$, $P = 0.001$).

Body Size Variation

In the 2003 season, male body length was not correlated with date ($r_{36} = -0.313$, $P = 0.056$), but male wing length decreased as the season progressed ($r_{36} = -0.519$, $P = 0.001$). Both female body length and female wing length, however, were not correlated with date ($r_{36} = -0.291$, $P = 0.077$, $r_{36} = -0.230$, $P = 0.164$; respectively). In the 2004 season, neither male body length, nor male wing length were correlated with date ($r_{32} = -0.074$, $P = 0.677$, $r_{32} = 0.577$, $P = 0.746$; respectively). In females, both body length and wing length were also not correlated with date ($r_{32} = -0.112$, $P = 0.527$, $r_{32} = -0.014$, $P = 0.938$; respectively).

Assortative Mating by Size

Table 1 summarises the calculated MA slopes between male and female body sizes for T and NT males found in copula, for seasons 2003 (Figs. 2 and 3)

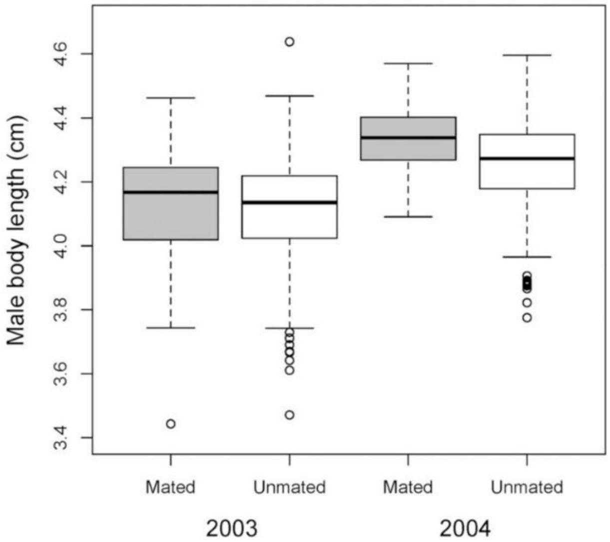

Fig. 1 Boxplot of male body length of mated (grey boxes) and unmated males (white boxes) in seasons 2003 and 2004. Medians, lower- and upper quartiles, sample minimums and maximums (whiskers), as well as atypical extreme values (open circles) are shown

and 2004 (Fig. 4). With the exception of the relationship exhibited by T males in 2003, and the relationship between male and female wing lengths in 2004, males and females of *Hetaerina americana* exhibit true assortative mating by size, since data points of the significant MA slopes exhibit homoscedasticity (r_s

Table 1 Slopes of major axis regressions (MA) of female body size as a function of male body size (BL = body length; WL = wing length; log_{10} transformed values), 95% confidence intervals (CIs) (when available), and their associated *P* values for territorial (T) and nonterritorial (NT) males of *Hetaerina americana*

Season 2003								
X	Y	Status	Slope	Lower - Upper CIs	MA *P* value	*n*	r_s	*P* value
Male BL	Female BL	Not considered	0.599	0.039–1.708	0.041	38	0.200	0.228
		T	2.494	NA – NA	0.332	18	0.122	0.627
		NT	0.635	0.075–1.769	0.036	11	−0.281	0.402
Male WL	Female WL	Not considered	2.304	1.461–4.499	0.001	38	−0.099	0.552
		T	2.432	1.274–8.671	0.012	18	−0.199	0.428
		NT	1.066	0.609–1.908	0.002	11	0.354	0.286
Season 2004								
X	Y	Status	Slope	Lower - Upper CIs	MA *P* value	*n*	*rho*	*P* value
Male BL	Female BL	Not considered	0.659	0.172–1.541	0.016	34	0.272	0.119
		T	0.595	0.155–1.314	0.015	29	0.247	0.197
Male WL	Female WL	Not considered	0.582	NA – NA	0.209	34	0.341	0.048
		T	0.618	NA – NA	0.123	29	0.458	0.012

Analyses in which male status was not considered are also shown (see Materials and Methods). Spearman's rank correlation (r_s) was used to test for heteroscedasticity of the absolute values of the residuals from MA regressions on the log_{10} transformed values of female size (see *Statistical analyses*). Non-significant correlations denote true assortative mating by size

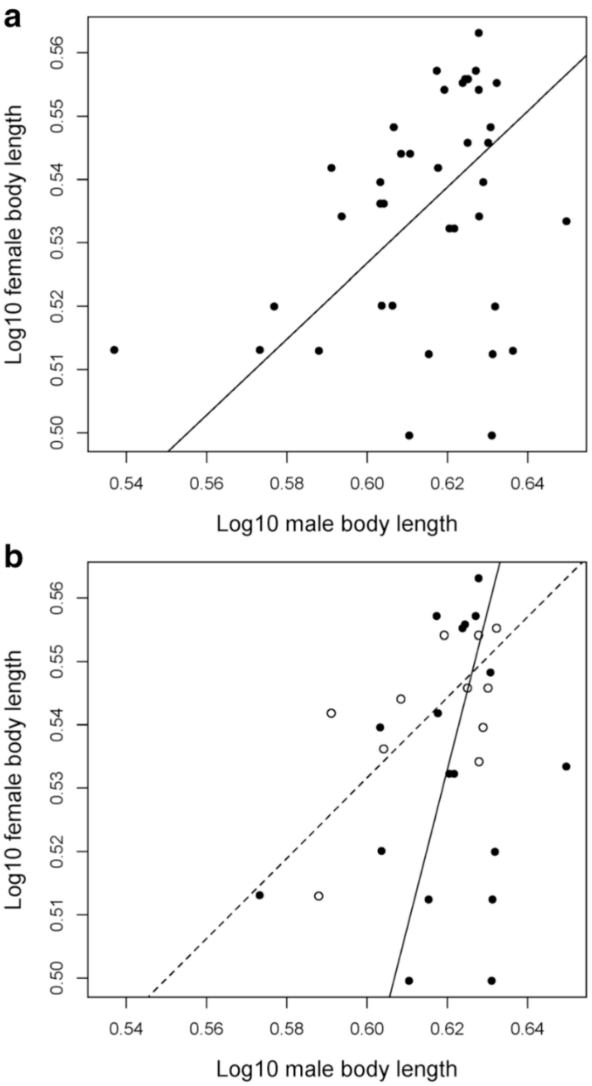

Fig. 2 Fitted major axis (MA) regression slopes between log_{10} female body length as a function of log_{10} male body length of copulating pairs of *Hetaerina americana*. **a** Full 2003 sample, including mated males with unknown status (T = territorial, NT = nonterritorial) ($n = 38$). The general pattern is consistent with true assortative mating by size [β, lower confidence interval (CI) – upper C]: 0.599, 0.039–1.708, $P = 0.041$ (Table 1). **b** 2003 subsample, including only mated males with known status. T males ($n = 18$): closed dots, continuous line; NT males ($n = 11$): open dots, dashed line). T males do not exhibit assortative mating by size, whilst NT males do (0.635, 0.075–1.769, $P = 0.036$)

statistic and relevant *P* values are provided in Table 1, for each MA regression). Interestingly, the majority of the significant slopes denote isometric relationships in the mating pattern between males and females; thus, males generally mate with females with body sizes similar to theirs.

The lineal models in which we tested for the influence of seasonality on the mating patterns between males and females, suggests that female body size is positively related to male body size, but not to the date when the mating pairs were collected (*Model 1*:

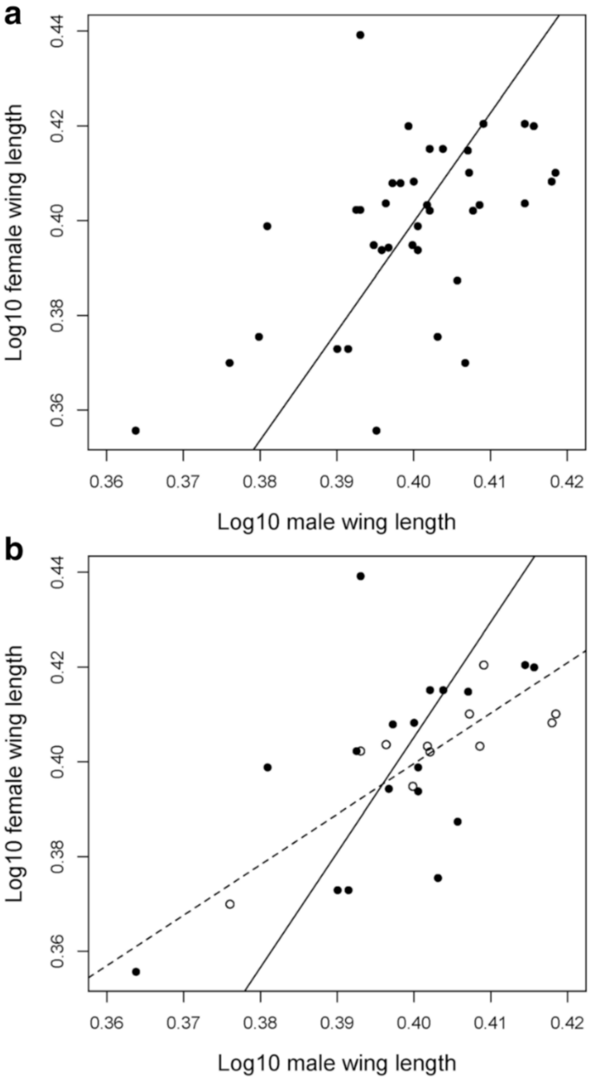

Fig. 3 Fitted MA regression slopes between log_{10} female wing length as a function of log_{10} male wing length of copulating pairs. **a** Full 2003 sample, including mated males with unknown status (T = territorial, NT = nonterritorial) ($n = 38$). The general pattern is consistent with true assortative mating by size [β, lower confidence interval (CI) – upper C]: 2.304, 1.461–4.499, $P = 0.001$ (Table 1). **b** 2003 subsample, including only mated males with known status. T males ($n = 18$): closed dots, continuous line; NT males ($n = 11$): open dots, dashed line). Both T and NT males exhibit true assortative mating by size (2.432, 1.274–8.671, $P = 0.012$; 1.066, 0.609–1.908, $P = 0.002$; respectively), albeit the hyperallometric slope exhibited by T males suggest larger-than-average males tend to mate with the largest females, compared to NT males, which exhibit an isometric slope

male body length, estimate ± SE, 0.265 ± 0.098, $t = 2.714$, $P = 0.008$; date, -0.001 ± 0.001, $t = 1.082$, $P = 0.283$; season, 0.002 ± 0.004, $t = 4.514$, $P = 0.001$; $F_{3,68} = 25.040$, $r^2 = 0.525$, $P = 0.001$; *Model 2*: male wing length, 0.559 ± 0.157, $t = 3.570$, $P = 0.001$; date, -0.001 ± 0.001, $t = 0.483$, $P = 0.631$; season, 0.007 ± 0.003, $t = 1.949$, $P = 0.055$; $F_{3,68} = 10.570$, $r^2 = 0.318$, $P = 0.001$).

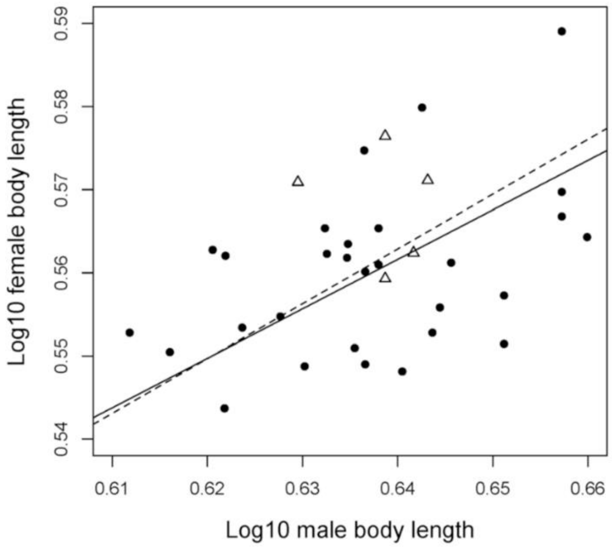

Fig. 4 Fitted MA regression slopes between log_{10} female body length as a function of log_{10} male body length of copulating pairs in 2004. The full sample ($n = 34$ males) includes mated males with unknown status (open triangles; $n = 5$). The general pattern, including all males (dashed line), is consistent with true assortative mating by size [β, lower confidence interval (CI) – upper C]: 0.659, 0.172–1.541, $P = 0.016$ (Table 1). The subsample, excluding males with unknown status (continuous line), suggests that true assortative mating by size occurs in T males (0.595, 0.155–1.314, $P = 0.015$)

Discussion

Our study has produced two major results. First, depending on the trait used to estimate body size, mated males are likely to be larger than unmated males. In any case, it appears that in *H. americana*, larger-than-average males are more likely to mate. Serrano-Meneses et al. (2007) showed that in this species, male body size was under directional selection, so that larger males had an advantage over smaller males, although Serrano-Meneses et al. (2007) did not test whether mated males were larger than unmated ones. Interestingly, body size does not differ amongst mated and unmated females. Potential explanations for these results are discussed below.

Our second main result suggests that true assortative mating by size is exhibited by *H. americana* and that it is not a by-product of seasonality (e.g. a temporal constraint), since i) data from both seasons were analysed separately, ii) the body sizes of males and females did not change considerably within seasons, and iii) female body size is positively related to male body size, but not to the date when the copulating pairs were observed. Thus, it is likely that the pattern is the result of the characteristic intense male-male competition of the species (Córdoba-Aguilar et al. 2009) – through the advantages of large male size – and perhaps, a process of male choice. Larger-than-average males in this species are better at defending territories compared to smaller males (Serrano-Meneses et al. 2007), since territory tenure and fighting rate increase with body size. This is possible since metabolic fat, the main energy source used during flight, increases with body size (Serrano-Meneses et al. 2007). Such males may further signal their condition and their ability to defend a territory through wing pigmentation (Grether 1996a; Contreras-Garduño et al. 2006; but see Contreras-

Garduño et al. 2007), since this trait increases hyperallometrically with body size (Álvarez et al. 2013). Nonetheless, the advantages of large female body size are not clear in this species. Serrano-Meneses et al. (2007) investigated whether female body size was related to fecundity, but did not find a relationship between egg size, egg number, and female body size. A positive relationship between female body size and fecundity (e.g. number of ovarioles) is expected in insects (Honěk 1993). In Odonata, this relationship has not been thoroughly investigated. Nonetheless, data from two damselfly species, *Coenagrion puella* (Banks and Thompson 1987) and *Ischnura graellsii* (Cordero 1991), show that fecundity is a positive function of female body size: in the former species, larger females produce more eggs because they live longer than smaller females, and in the latter, clutch size increases with female body size. Unlike Banks and Thompson (1987) and Cordero (1991), Serrano-Meneses et al. (2007) did not investigate lifetime egg production, or clutch size, but rather "instant fecundity", since they measured and counted the number of mature eggs within the abdomens of dead, collected females. Thus, we believe that it is likely that males increase their fitness by mating with large females, if such females are more fecund. Smaller males may not be able to copulate with large females, because of mechanical or female behavioural constraints (male damselflies cannot force females to copulate; Corbet 1963), and could then have no alternative but to mate with smaller females. This may explain why those males found in copula were larger than those males never seen copulating.

Finally, we suggest that the possible role of male choice in assortative mating by size in *H. americana* ought to be considered. A dominant view on the process of animal reproduction is one in which indiscriminating males compete over access to choosy females (Edward and Chapman 2011). Thus, much research has focused on how evolutionary change is modelled by male-male competition and female choice (see Andersson 1994). More recent studies have shown, however, that reproduction is not a harmonious, simple process (reviewed by Arnqvist and Rowe 2005), but that the roles of males and females are dynamic and variable (Edward and Chapman 2011). Several species are characterised by males exerting mate choice and females competing over access to males ("reversed" sex roles), and although this is especially likely to occur in species in which males contribute to parental care (Gwynne 1991), this behaviour has also been documented in species in which males do not contribute substantially to parental care (Amundsen 2000; Bonduriansky 2001). We suggest that males of *H. americana* may be able to distinguish the reproductive potential of females, and thus may aim to copulate with those females with the highest reproductive value (i.e. large females). Nonetheless, we currently do not have data to test this. A difficulty is the general assumption that males of this species do not discriminate amongst females, so they are assumed to be eager to mate with any female (e.g. Grether 1996a, b; Córdoba-Aguilar and Cordero-Rivera 2005; Serrano-Meneses et al. 2007). However, the idea that sperm may be costly to produce, and that males may allocate more sperm to females of superior reproductive quality (e.g. Wedell et al. 2002) has not been tested or even considered to occur in this or other *Hetaerina* species. In dung flies, for instance, males seem to modify their sperm allocation in relation to the perceived female's fecundity (Parker and Pizzari 2010). We believe that much would be gained from investigating whether males in fact evaluate females and exert choice, as our current results suggest.

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Compliance with Ethical Standards

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

References

Álvarez HA, Serrano-Meneses MA, Reyes-Márquez I, Jiménez-Cortés JG, Córdoba-Aguilar A (2013) Allometry of a sexual trait in relation to diet experience and alternative mating tactics in two rubyspot damselflies (Calopterygidae: *Hetaerina*). Biol J Linn Soc 108:521–533

Amundsen T (2000) Why are female birds ornamented? Trends Ecol Evol 15:149–155

Andersson M (1994) Sexual selection. In: Princeton University press. USA, New Jersey

Arnqvist G, Rowe L (2005) Sexual conflict. Princeton University press, New Jersey, USA

Arnqvist G, Rowe L, Krupa JJ, Sih A (1996) Assortative mating by size: a meta-analysis of mating patterns in water striders. Evol Ecol 10:265–284

Banks MJ, Thompson DJ (1987) Lifetime reproductive success of females of the damselfly *Coenagrion puella*. J Anim Ecol 56:815–832

Black JM, Owen M (1995) Reproductive performance and assortative pairing in relation to age in barnacle geese. J Anim Ecol 64:234–244

Bernstein R, Bernstein S (2003) Assortative mating in insects: sexual selection vs. sampling artifact. West N Am Nat 63:374–381

Bonduriansky R (2001) The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol Rev 76:305–339

Bonneaud C, Chastel O, Federici P, Westerdahl H, Sorci G (2006) Complex *Mhc*-based mate choice in a wild passerine. Proc R Soc B 273:1111–1116

Brown WD (1990) Size-assortative mating in the blister beetle *Lytta magister* (Coleoptera: Meloidae) is due to male and female preference for larger mates. Anim Behav 40:901–909

Contreras-Garduño J, Buzatto BA, Abundis L, Nájera-Cordero K, Córdoba-Aguilar A (2007) Wing colour properties do not reflect male condition in the American rubyspot (*Hetaerina americana*). Ethology 113: 944–952

Contreras-Garduño J, Canales-Lazcano J, Córdoba-Aguilar A (2006) Wing pigmentation, immune ability, fat reserves and territorial status in males of the rubyspot damselfly, *Hetaerina americana*. J Ethol 24:165–173

Corbet PS (1963) A biology of dragonflies. In: Quadrangle Books. Chicago, USA

Corbet PS (1999) Dragonflies: behaviour and ecology of Odonata. Harley Books, Martins, United Kingdom

Cordero A (1991) Fecundity of *Ischnura graellsii* (Rambur) in the laboratory (Zygoptera: Coenagrionidae). Odonatologica 20:37–44

Córdoba-Aguilar A (1994) Male substrate use in relation to age and size in *Hetaerina cruentata* (Rambur) (Zygoptera: Calopterygidae). Odonatologica 23:399–403

Córdoba-Aguilar A, Cordero-Rivera A (2005) Evolution and ecology of Calopterygidae (Zygoptera: Odonata): status of knowledge and research perspectives. Neotrop Entomol 34:861–879

Córdoba-Aguilar A, Raihani G, Serrano-Meneses MA, Contreras-Garduño J (2009) The lek mating system of *Hetaerina* damselflies (Insecta: Calopterygidae). Behaviour 146:189–207

Crespi BJ (1989) Causes of assortative mating in arthropods. Anim Behav 38:980–1000

Edward DA, Chapman T (2011) The evolution and significance of male mate choice. Trends Ecol Evol 26: 647–654

Fairbairn DJ (1988) Sexual selection for homogamy in the Gerridae: an extension of Ridley's comparative approach. Evolution 42:1212–1222

Fairbairn DJ (1997) Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. Annu Rev Ecol Syst 28:659–687

Fauchier J, Thomas F (2001) Interaction between *Gammarinema gammari* (Nematoda), *Microphallus papillorobustus* (Trematoda) and their common host *Gammarus insensibilis* (Amphipoda). J Parasitol 87:1479–1481

Forbes MRL, Pagola H, Baker RL (1992) Causes of a non-random pairing by size in the brine shrimp, *Artemia salina*: (Crustacea: Anostraca). Oecologia 91:214–219

García-Navas V, Ortego J, Sanz JJ (2009) Heterozygosity-based assortative mating in blue tits (*Cyanistes caeruleus*): implications for the evolution of mate choice. Proc R Soc B 276:2931–2940

Grether GF (1996a) Sexual selection and survival selection on wing coloration and body size in the rubyspot damselfly *Hetaerina americana*. Evolution 50:1939–1948

Grether GF (1996b) Intrasexual competition alone favors a sexually dimorphic ornament in the rubyspot damselfly *Hetaerina americana*. Evolution 50:1949–1957

Gwynne DT (1991) Sexual competition among females: what causes courtship-role reversal. Trends Ecol Evol 6:118–121

Harvey PH, Mace GM (1982) Comparisons between taxa and adaptive trends: problems of methodology. In: King's College Sociobiology Group (ed) Current problems in sociobiology. Cambridge University Press, New York, USA, pp 343–361

Honěk A (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos 66: 483–492

Hooper PL, Miller GF (2008) Mutual mate choice can drive costly signaling even under perfect monogamy. Adapt Behav 16:53–70

Jawor JM, Linville SU, Beall SM, Breitwisch R (2003) Assortative mating by multiple ornaments in northern cardinals (*Cardinalis cardinalis*). Behav Ecol 14:515–520

Jiang Y, Bolnick DI, Kirkpatrick M (2013) Assortative mating in animals. Am Nat 181:E125–E138

Kemp DJ (2008) Female mating biases for bright ultraviolet iridescence in the butterfly *Eurema hecabe* (Pieridae). Behav Ecol 19:1–8

Koskimäki J, Rantala MJ, Taskinen J, Tynkkynen K, Suhonen J (2004) Immunocompetence and resource holding potential in the damselfly, *Calopteryx virgo* L. Behav Ecol 15:169–173

Krüger O, Lindström J, Amos W (2001) Maladaptive mate choice maintained by heterozygote advantage. Evolution 55:1207–1214

LaBarbera M (1989) Analyzing body size as a factor in ecology and evolution. Annu Rev Ecol Syst 20:97–117

Lawrence WS (1986) Male choice and competition in *Tetraopes tetraophthalmus*: effects of local sex ratio variation. Behav Ecol Sociobiol 18:289–296

Marden JH, Waage JK (1990) Escalated damselfly territorial contests are energetic wars of attrition. Anim Behav 39:954–959

Masumoto T (1999) Size assortative mating and reproductive success of the funnel-web spider, *Agelena limbata* (Araneae; Agelenidae). J Insect Behav 12:353–361

McArdle BH (1988) The structural relationship: regression in biology. Can J Zool 66:2329–2339

McLain DK, Shure DJ (1987) Pseudocompetition: interspecific displacement of insect species through misdirected courtship. Oikos 49:291–296

Parker GA, Pizzari T (2010) Sperm competition and ejaculate economics. Biol Rev 85:897–934

Partridge L (1983) Non-random mating and offspring fitness. In: Bateson P (ed) Mate choice. Cambridge University Press, Cambridge, United Kingdom, pp 227–256

Pavković-Lučić S, Kekić V, Čvoro A (2009) Larger male mating advantage depends on the sex ratio in *Drosophila melanogaster*. Ethol Ecol Evol 21:155–160

Plaistow S, Siva-Jothy MT (1996) Energetic constraints and male mate-securing tactics in the damselfly *Calopteryx splendens xanthostoma* (Charpentier). Proc R Soc B 263:1233–1239

R Core Team (2018) R: A language and environment for statistical Computing R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Raihani G, Serrano-Meneses MA, Córdoba-Aguilar A (2008) Male mating tactics in the American rubyspot damselfly: territoriality, nonterritoriality and switching behaviour. Anim Behav 75:1851–1860

Ramírez-Delgado J, López-García K, Lara C, Serrano-Meneses MA (2015) Wing pigmentation in males of a territorial damselfly: alternative reproductive tactics, allometry and mating success. J Insect Behav 28: 569–581

Ridley M (1983) The explanation of organic diversity: the comparative method and adaptations for mating. Clarendon Press, Oxford

Serrano-Meneses MA, Córdoba-Aguilar A, Azpilicueta-Amorín M, González-Soriano E, Székely T (2008) Sexual selection, sexual size dimorphism and Rensch's rule in Odonata. J Evol Biol 21:1259–1273

Serrano-Meneses MA, Córdoba-Aguilar A, Méndez V, Layen SJ, Székely T (2007) Sexual size dimorphism in the American rubyspot: male body size predicts male competition and mating success. Anim Behav 73: 987–997

Sharp NP, Agrawal AF (2009) Sexual selection and the random union of gametes: testing for a correlation in fitness between mates in *Drosophila melanogaster*. Am Nat 174:613–622

Sokal RR, Rohlf FJ (2012) Biometry: the principles and practice of statistics in biological research. W. H. In: Freeman and company. USA, New York

Tárano Z, Herrera EA (2003) Female preferences for call traits and male mating success in the neotropical frog *Physalaemus enesefae*. Ethology 109:121–134

Warton DI, Duursma RA, Falster DS, Taskinen S (2012) Smatr 3- an R package for estimation and inference about allometric lines. Methods Ecol Evol 3:257–259

Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. Biol Rev 81: 259–291

Wedell N, Gage MJG, Parker GA (2002) Sperm competition, male prudence and sperm-limited females. Trends Ecol Evol 17:313–320

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Seasonal variation in the allometry of the pigmentation of the wings in adult males of the territorial devil's horse *Hetaerina vulnerata* (Insecta: Odonata)

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Seasonal variation in the allometry of wing pigmentation in adult males of the territorial damselfly *Hetaerina vulnerata* (Insecta Odonata)

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Several sexually selected traits exhibit positive allometry. One of the explanations for this pattern suggests that positive allometry should be expected only for "pure" threat male signals, whilst isometry and negative allometry should occur in "pure" courtship signals. Wing pigmentation (WP) is a sexually selected trait exhibited by the males of several damselfly taxa (Odonata Zygoptera), which has been the focus of a number of studies of allometry. Whilst WP tends to exhibit positive allometry in many taxa, the pattern is not general. Previous studies have shown that in *Hetaerina vulnerata*, a territorial species in which WP is used as a "pure" threat signal, positive allometry is common. However, little attention has been paid to the seasonal variation in the allometry of WP in this species. To address this research gap, we collected data from a full reproductive season, from a natural population of *H. vulnerata* located in Central Mexico. Our results suggest that, first, male WP and body size generally increase throughout the season. Second, WP increases as the end of the season approaches, independently of body size. Finally, WP tends to be either not related to body size, or to exhibit isometry during the first half of the season (April–June), and positive allometry during the second half of the season (July–October). We suggest that our results may be explained by (i) the availability of resources critical to the development of larvae, as well as the duration of development, and (ii) an increase in the number or larger males towards the end of the season, which may favour the exaggeration of WP. Furthermore, the opportunity for sexual selection may vary, so that WP may be more relevant to male mating success towards the end of the reproductive season.

KEY WORDS: allometry, damselfly, seasonal variation, wing pigmentation.

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INTRODUCTION

Allometry is described as any disproportional change in a morphological trait scaled against body size (Eberhard 2009). It is thus measured using the equation $y = \alpha x^{\beta}$, where β is the slope of a linear regression between the trait of interest (y) and body size (x) (Bonduriansky 2007). When $\beta = 1$, the size of the trait increases at the same rate as body size (i.e. isometry); when $\beta < 1$, the size of the trait increases at a lower rate than body size (i.e. negative allometry); and finally, when $\beta > 1$, the size of the trait increases at a higher rate than body size (i.e. positive allometry) (Huxley & Teissier 1936). A positively allometric trait will be large – relative to body size – in large individuals, whereas it will be disproportionately small in smaller individuals. Several traits, including sexual traits, exhibit positive allometry (Kodric-Brown et al. 2006). Examples of this are the forceps of earwigs (Simmons & Tomkins 1996), the eyestalks of diopsid flies (Wilkinson & Dodson 1997; Worthington et al. 2012), the horns of dung beetles (Tomkins et al. 2005; McCullough et al. 2015), and the sex combs of fruit flies (Sharma et al. 2011). This pattern has attracted the attention of researchers for the past decades, mainly because the study of the underlying causes of allometry allow us to further understand resource allocation conflicts (Kodric-Brown et al. 2006), and patterns of sexual and viability selection acting on both, the traits of interest and body size (Cotton et al. 2004; Bonduriansky 2007; Álvarez et al. 2013).

One of the explanations for the allegedly general positive allometry of sexual traits (Kodric-Brown et al. 2006) is the "positive allometry hypothesis" (Bonduriansky 2007). The hypothesis has two main assumptions, which are assumed to lead to the evolution of positive allometry of sexual traits: (i) male traits, such as signals and weapons, are under directional sexual selection, and (ii) body size is related to condition (Green 1992; Petrie 1992; Bonduriansky 2007). Thus, large males bearing an overly exaggerated sexual trait (under strong directional sexual selection) will be preferred by females, whereas small males will experience lower mating success, given that the expression of their traits may be penalised by viability costs (Bonduriansky 2007). However, not all sexual traits exhibit positive allometry (Bonduriansky & Day 2003; Kodric-Brown et al. 2006; Bonduriansky 2007), which has led to disagreements regarding the allometry of such traits. A possible solution is provided by Eberhard et al. (2018), who analysed the allometry of sexual traits according to their function. Thus, Eberhard et al. (2018) distinguished between traits that were used as "pure" courtship (male-female signals), or as "pure" threat signals (male-male signals, excluding weapons). Their results suggest that sexual selection favours negative allometries on courtship traits and positive allometries on threat traits. This may occur because even though courtship signals do convey information on an individual's condition, this condition does not need to relate to the allometry of the trait (Petrie 1988; West-Eberhard 2014). Threat signals, in contrast, may be honest or dishonest signals used by males to increase and transmit their perceived size and power ("I am larger and more powerful than you"; Eberhard et al. 2018). Additionally, the benefits from winning a contest may be greater for larger males, given that these usually compete over high-quality situations or locations, which may significantly enhance their reproductive success. As a result, larger males may commonly compete with similar sized individuals. If large males bear large signals, two contesting males may find it difficult to adequately judge the differences between their signals (Weber's Law in sensory physiology; Marks 1974). The larger the male, the larger the signal should be in order for the rival to perceive the difference between their signals (reviewed by Eberhard et al. 2018).

Amongst damselflies (Odonata Zygoptera), wing pigmentation (WP) is a conspicuous male trait that has been the focus of attention of a number of studies of allometry. The males of several species exhibit pigmented spots on the wings, often in a wide range of colours and patterns (see Corbet 1999). Male WP is known to relate to sexual selection in several damselflies (reviewed by Córdoba-Aguilar & Cordero-Rivera 2005; Suhonen et al. 2008), although most of the studies have focused on species of the genera *Calopteryx* and *Hetaerina* (Calopterygidae). The same relationship has rarely been investigated in dragonflies (e.g. Moore 1990; Córdoba-Aguilar & Méndez 2006; Contreras-Garduño et al. 2011). A previous study, which investigated the allometry of WP in 14 species of Calopterygidae, found a mixture of isometry and positive allometry (Outomuro et al. 2014), with no clear distinction between species exhibiting intrasexual or intra- and intersexual selection (but see Discussion). Other studies have investigated whether the allometry of WP differs between territorial and non-territorial males of *H. americana* and *H. vulnerata* (Álvarez et al. 2013; Ramírez-Delgado et al. 2015), and found that although WP exhibited mostly positive allometry, the slopes were not different amongst the alternative reproductive tactics. Ramírez-Delgado et al. (2015) further compared the slopes of mated and unmated males of *H. vulnerata*, and since these were not statistically different, Ramírez-Delgado et al. (2015) suggested that the positive allometry of WP may not be selectively advantageous per se. Nonetheless, these studies have not considered the seasonal variation in the allometry of WP. This is important, because the expression of sexual and non-sexual traits is expected to vary throughout the year, given that environmental conditions are not constant (e.g. Plaistow et al. 2005). This shortfall was considered by Córdoba-Aguilar et al. (2010), who tested how the allometry of WP in *H. americana* varied in two periods within a single year (January–April vs July–October 2007). Their results showed that WP exhibited positive allometry with respect to head width, and only in the late period of the year, whilst the rest of their results showed that WP was unrelated to body size. Nonetheless, considering the surprising non-significant results reported, and the limitations of the analytical approach and sample sizes used by Córdoba-Aguilar et al. (2010), we consider that the seasonal variation in the allometry of WP in *Hetaerina* (or Calopterygidae) has not been thoroughly explored.

In the present study, we investigated the seasonal variation in the allometry of WP in the damselfly *H. vulnerata*, by using data collected throughout the full reproductive period of a natural population of this species, located in the Central Mexican Plateau. The males of this species exhibit non-fixed reproductive tactics (e.g. Ramírez-Delgado et al. 2015). Territorial males establish and defend territories along the shores of streams, since the possession of a territory determines higher-than-average male mating success (Alcock 1987; Ramírez-Delgado et al. 2015). Non-territorial males, instead, wander along the shores of the stream and do not acquire a territory; thus, their mating success is lower than that of territorial males (Ramírez-Delgado et al. 2015). Both territorial and non-territorial males exhibit a bright red spot at the base of the wings, a threat signal which is known to evolve exclusively via sexual selection in the closely related *H. americana* (Grether 1996a) (courtship is absent in *Hetaerina* damselflies). Here, by measuring WP and body size as either lengths or areas, we predicted WP to exhibit (i) variation throughout the reproductive season, and particularly (ii) positively allometric slopes, which will be steeper towards the second half of the year, given the long reproductive season of the species (Ramírez-Delgado et al. 2015), which may allow more time for the larvae to develop and to acquire the necessary resources to achieve good body conditions.

MATERIAL AND METHODS

Adult *Hetaerina vulnerata* males were collected using aerial entomological nets, along a 200 m section of a stream in San Tadeo Huiloapan, Panotla, Tlaxcala, Mexico (19°23′42.5″N, 98°15′36″W, approximately 2450 m asl). The stream crosses a section of small-scale corn crops and oak woodland. Sampling started at 11:00 and finished at 14:00 hr (Central Standard Time); this is the period when *Hetaerina* damselflies show the highest activity (see Grether 1996b; Ramírez-Delgado et al. 2015). The collection of males was performed once per month, from April to October 2016, within the period in which individuals of this species are present around the area. Sampling effort was similar throughout the reproductive season: we searched for, marked and photographed males for approximately 3 hr each sampling day. No data were obtained for August, given strong social unrest that prevented us from entering the study site. Once a male was captured, it was marked with a unique number (from one to three digits) on the left hindwing, using the fine side of a Sharpie® Twin Tip black permanent marker (see similar methodologies in Serrano-Meneses et al. 2007 and Álvarez et al. 2013). With this, we made sure an individual was not measured twice. The animal was placed dorsally on a graph paper clipping, holding all four wings with microscope slides, on top of a Petri dish. Three photographs were taken per male at a constant distance (for later repeatability measurements; see below), with a Canon EOS 50D reflex digital camera, fitted with a 60 mm Canon macro lens. Handling time was approximately 2 min per male. All males were released immediately after manipulation.

The age of each male was estimated from morphological cues (see Córdoba-Aguilar 1994; Ramírez-Delgado et al. 2015): (i) juvenile mature individuals have a bright, intense body colouration and highly transparent wings; (ii) mature individuals show less brightness and intensity in body colouration and have less transparent wings; (iii) old individuals have dark body colouration and the tips of their wings are usually torn or missing. In our study, we only included individuals of age class two, since those belonging to class one may not have fully developed wing spots (Contreras-Garduño et al. 2007), and males from class three occasionally have damaged wings, which may potentially bias body size estimates (Córdoba-Aguilar et al. 2010).

Image analyses were carried out using the software ImageJ ver. 1.8.0 (Schneider et al. 2012). From the photographs, a single person (F.E. Rodríguez-Escobar) obtained the following data from left forewings: (a) wing length and (b) wing area (cm; as proxies of body size), and (c) WP length and (d) WP area (cm^2; as proxies of WP). This was done in order to minimise measurement error variance. Body size and WP were estimated in this way in order to facilitate comparison with other studies on the allometry of WP in damselflies (e.g., Bello-Bedoy et al. 2015; Ramírez-Delgado et al. 2015), which have analysed these traits as either lengths or areas. Wing length was measured from the insertion of the wing to the thorax to the tip of the wing; and WP length was measured following an imaginary straight line over the posterior cubital vein, from the insertion of the wing to the thorax, to the distal end of the pigmented patch (see a similar approach in Álvarez et al. 2013). From each photograph taken, we estimated the length or area per male. These three values were averaged to obtain either body size or WP.

Statistical analyses

To assess the consistency of our measurements, we calculated the intraclass correlation coefficients (ICCs; repeatability), as recommended by Wolak et al. (2012). Individual measurements from each photograph were analysed using the R (R Core Team 2018; ver. 3.5.1 “Feather Spray”) package “ICC” (Wolak et al. 2012). Our measurements, as suggested by the 95% ICCs obtained for each trait, were highly repeatable: age (ICC, lower CI–upper CI; n = 117, k = 3, in all cases): 0.953, 0.937–0.966; wing length: 0.980, 0.973–0.986; wing area: 0.999, 0.998–0.999; WP length: 0.973, 0.962–0.980; WP area: 0.991, 0.987–0.994.

We investigated seasonal changes in body size and WP in two ways. First, we used ordinary least squares regressions (OLS). In these analyses, either trait (i.e. wing length, wing area, WP length or WP area) was used as the dependent variable, and the months when males were measured (henceforth *date*) as the independent variable. To validate the use of OLS, we assessed the homoscedasticity of residuals of each regression through the use of normality plots (Q–Q plots; Sokal & Rohlf 2003). Under this graphical method, a variable is normally distributed if the expected and observed values follow a straight line (e.g. Álvarez et al. 2013). The assumption of homoscedasticity of residuals was not violated by our tests. Second, since we were unable to obtain data on 1 month (August), and OLS can be sensitive to missing fixed values on the x-axis, we corroborated our results using ANOVA tests. In these analyses, either trait was used as an independent variable, and *date* as a six-level factor. Pair-wise comparisons after a significant ANOVA tests were carried out using Tukey's HSD post hoc tests.

Second, we investigated the relationship between our estimates of WP (WP length vs WP area) and body size (wing length vs wing area) using Pearson's moment product correlations.

Finally, to investigate a general pattern of change in WP throughout the season, we used two Generalised Linear Models (GLMs). In these analyses, we used either WP length or area as dependent variables, *date* as a six-level factor, and wing length or area as independent variables, respectively. We included body size in these analyses in order to control for its confounding effects on the seasonal changes of WP (WP and body size are correlated; see Results). The interaction term *date**body size was tested in both models, and since it was not statistically significant (WP length model interaction term estimate ± SE: 0.038 ± 0.022, $t_{113} = 1.797$, $P = 0.075$; WP area model: 0.002 ± 0.006, $t_{113} = 0.278$, $P = 0.781$), it was removed from the analyses. Note that these analyses do not estimate the allometry of WP, since special tests are necessary to summarise the allometric relationships between pairs of variables (see below).

Estimation of the allometry of WP

To investigate the allometric relationship between WP and body size we used major axis regressions (MA). This method is preferred over others, such as OLS (Model I regression) and reduced major axis regression (RMA) because of a number of advantages (Harvey & Pagel 1991; Sokal & Rohlf 2003; Warton et al. 2006; Warton 2007). In brief, MA regression can be used to describe a line-of-best-fit between two continuous variables, whilst assuming that both x and y variables are estimated with error, and that error variance is equal in both variables. It further allows the statistical testing of the general null hypothesis $\beta = 0$, and the null hypothesis of isometry $\beta = 1$ (McArdle 1988; LaBarbera 1989; Fairbairn 1997). The method performs better if both variables are transformed to a logarithmic scale (Sokal & Rohlf 2003); thus, data on WP and body size were $\log_{10}$ transformed prior to analyses. WP was analysed as a function of body size for each month of the season. To validate the use of MA, we plotted the residuals of each regression against their axis scores. In no case, we found a pattern, which suggests that the variables are linearly related, and that residuals have the same variance at all points along the fitted line (Miller 1986; Warton et al. 2006; Warton 2007).

MA regressions were carried out using the "smatr" package (Warton et al. 2012). A slope is considered different from $\beta = 1$ if the calculated confidence intervals (CIs) exclude $\beta = 1$ and the associated *P*value of the regression is ≤ 0.05. "smatr" implements a procedure to test whether a calculated slope differs from a given slope value ("slope test"; see Warton et al. 2006 for a description). We used the procedure to test whether all significant slopes were $\beta = 1$.

All statistical analyses and figures were produced using R (R Core Team 2018; ver. 3.5.1 "Feather Spray").

RESULTS

Seasonal changes in body size and WP

Our results suggest that wing length was unrelated to date ($\beta \pm$ SE; $\beta = 0.003 \pm 0.004$, $F_{1,115} = 0.720$, $r^2 = 0.006$, $P = 0.398$), whereas wing area ($\beta = 0.021 \pm 0.004$, $F_{1,115} = 21.210$, $r^2 = 0.156$, $P = 0.001$), WP length ($\beta = 0.005 \pm 0.002$, $F_{1,115} = 5.414$, $r^2 = 0.045$, $P = 0.021$) and WP area ($\beta = 0.005 \pm 0.001$, $F_{1,115} = 50.600$, $r^2 = 0.306$, $P = 0.001$) showed slight increases as the season progressed. These results were confirmed by ANOVA tests, since we found no differences in wing length across the season ($F_{5,111} = 1.417$, $P = 0.224$), whilst there were differences in wing area ($F_{5,111} = 4.963$, $P = 0.001$; Fig. 1A), WP length ($F_{5,111} = 6.292$, $P = 0.001$; Fig. 1B) and WP area ($F_{5,111} = 10.920$, $P = 0.001$; Fig. 1C) throughout the season. Post hoc pair-wise comparisons show that wing area (Tukey's HSD, April vs October, $P = 0.001$), WP length (April vs September, $P = 0.004$) and WP area (April vs October, $P = 0.001$) were smaller at the onset of the season than they were at the end.

The similar behaviour exhibited by the traits, which were estimated as lengths or areas, is most likely driven by the correlation between measurements (WP length vs WP

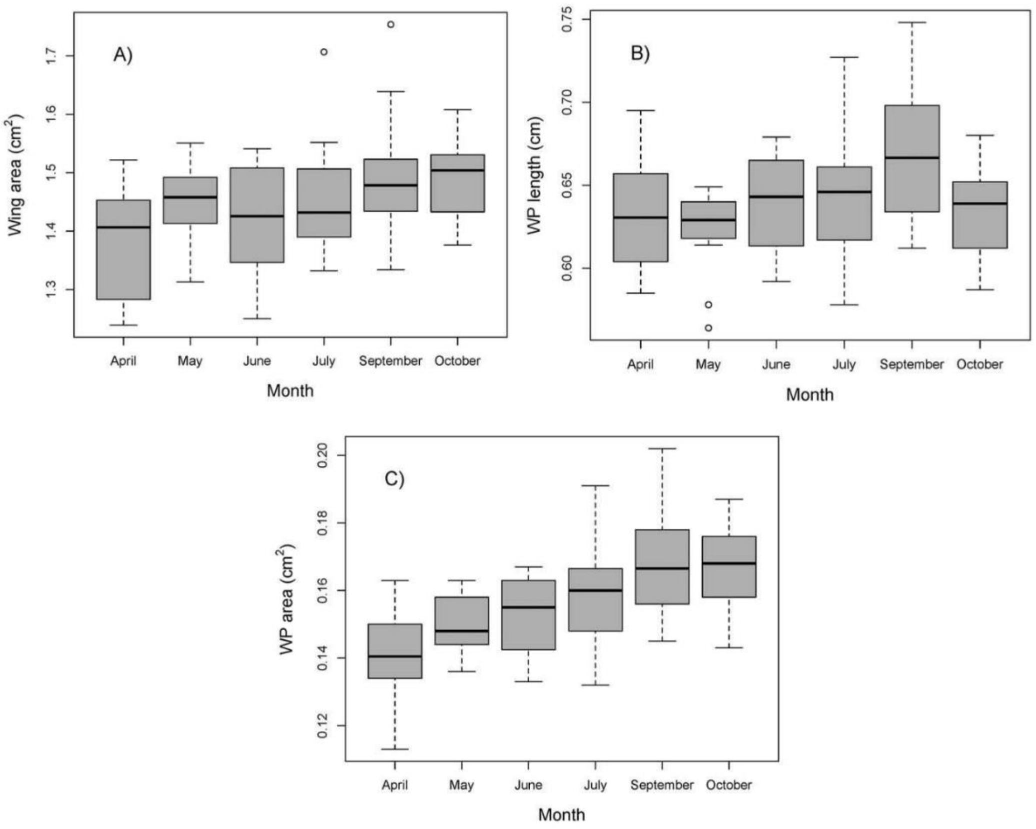

Fig. 1. — Boxplots of adult *Hetaerina vulnerata* male traits showing significant seasonal variation during 2016. (A) Wing area (cm^2), (B) WP length (cm), and (C) WP area (cm^2) are shown as a function of the months in which we were able to access the study site. Medians, lower- and upper quartiles, sample minimums and maximums (whiskers), as well as atypical extreme values (open circles) are shown.

area, Pearson's product moment correlation, $r_{115} = 0.802$, $P = 0.001$; wing length vs wing area, $r_{115} = 0.762$, $P = 0.001$).

Finally, WP length (GLM; estimate ± SE: date, 0.004 ± 0.002, $t_{114} = 2.246$, $P = 0.027$; wing length, 0.273 ± 0.035, $t_{114} = 7.827$, $P = 0.001$) and WP area (*date*, 0.003 ± 0.001, $t_{114} = 5.014$, $P = 0.001$; wing area, 0.117 ± 0.010, $t_{114} = 11.257$, $P = 0.001$) increase with *date*, when body size is controlled for. Thus, males emerging towards the end of the season tend to exhibit disproportionately larger-than-average pigmented spots.

Allometry of WP

Tables 1 and 2 summarise the calculated MA slopes between WP length and wing length (lengths), and WP area and wing area (areas), respectively. When *date* is not considered (i.e. when the full dataset is analysed), WP exhibits positive allometry, regardless of whether the traits are estimated as lengths (Fig. 2) or areas (Fig. 3). When *date* is considered, WP is (i) either not related to body size in April and May for lengths, or exhibits isometry in the same months for areas, (ii) isometry in June, and (iii) positive allometry in July, September and October. Note that the allometric relationships between WP and body size change similarly for lengths and areas from June until the end of the season.

DISCUSSION

Notwithstanding the relatively limited sample size, this study offers valuable insights into the seasonal variation of WP in *Hetaerina vulnerata*, which can be summarised in three main results. First, male WP and body size generally increase

Table 1.

Slopes, 95% confidence intervals (CIs), r^2 and P values obtained from MA regressions (see Material and Methods) fitted between the $\log_{10}$ transformed values of WP length and wing length of adult males of *Hetaerina vulnerata* throughout 2016. "P value" refers to the significance of the MA regressions between traits. "Slope test P value" refers to a procedure in which we tested whether the calculated slope was significantly different from $\beta = 1$ (see Warton et al. 2006). Where the relationship was not significant ($P > 0.05$), the slope test procedure was not executed.

Period	Slope	Lower CI, Upper CI	r^2	P value	Slope test P value	n
Full season	2.987	2.358, 3.999	0.339	0.001	0.001	117
April	2.787	0.872, – 6.023*	0.180	0.130	Not tested	14
May	– 44.182	3.931, – 3.299†	0.003	0.851	Not tested	13
June	2.059	0.936, 11.598	0.312	0.024	0.067	16
July	2.309	1.431, 4.752	0.476	0.001	0.002	19
September	2.613	1.835, 4.233	0.489	0.001	0.001	30
October	3.009	1.539, 15.246	0.218	0.019	0.003	25

Notes: *the CIs include the y axis — the actual CIs are (0.872, infinity) and (– infinity, – 6.023); †the calculated non-significant MA slope falls within the range of the CIs, as these include the y axis — the actual CIs are (3.931, infinity) and (– infinity, – 3.299).

Table 2.

Slopes, 95% confidence intervals (CIs), r^2 and P values obtained from MA regressions fitted between the $\log_{10}$ transformed values of WP area and wing area of adult males throughout 2016. “P value” denotes the significance of the MA regressions between traits. “Slope test P value” refers to a procedure in which we tested whether the calculated slope was significantly different from $\beta = 1$.

Period	Slope	Lower CI, Upper CI	r^2	P value	Slope test P value	n
Full season	1.866	1.616, 2.180	0.607	0.001	0.001	117
April	1.655	0.920, 3.758	0.518	0.004	0.083	14
May	1.364	0.782, 2.679	0.594	0.002	0.230	13
June	1.250	0.942, 1.690	0.802	0.001	0.112	16
July	1.827	1.164, 3.328	0.530	0.001	0.012	19
September	1.833	1.326, 2.718	0.558	0.001	0.001	30
October	2.220	1.310, 5.079	0.348	0.002	0.005	25

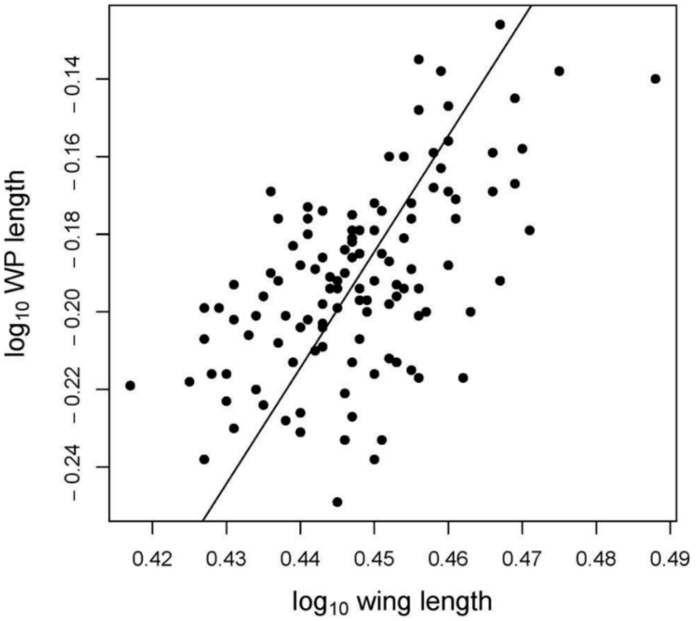

Fig. 2. — Major axis regression slope fitted between $\log_{10}$ WP length and $\log_{10}$ wing length of males measured throughout the full 2016 season. WP length exhibits positive allometry [β, lower confidence interval (CI), upper CI; 2.987, 2.358, 3.999, $P = 0.001$].

as the season progresses. Second, there is a general, steady increase in the expression of WP as the end of the season approaches, which is independent of body size. Finally, our analyses of the allometry of WP suggest that, depending on whether WP and body size are calculated as lengths or areas, from April until June WP tends to be either not related to body size, or to exhibit isometry. From July to October, WP exhibits positive allometry. We discuss potential explanations to our results below.

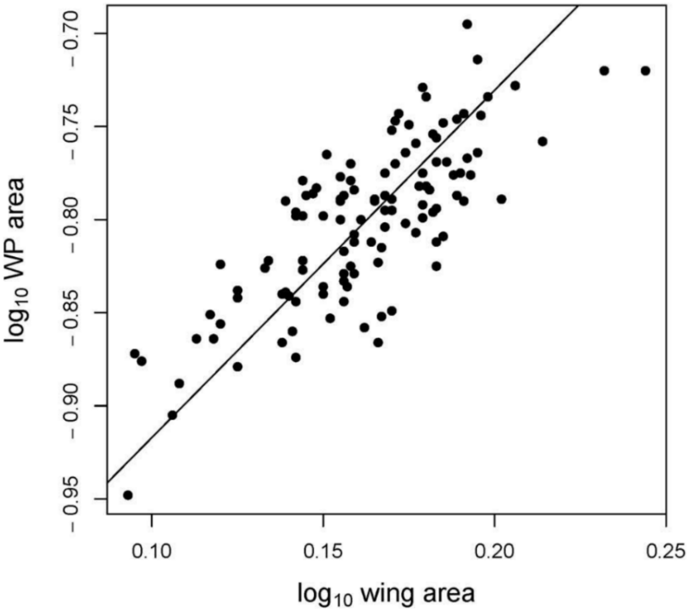

Fig. 3. — Major axis regression slope fitted between log$_{10}$ WP area and log$_{10}$ wing area of males measured throughout 2016. As it occurs with lengths, WP area exhibits positive allometry [β, lower confidence interval (CI), upper CI; 1.866, 1.616, 2.180, $P = 0.001$].

First, the body size of adult damselflies commonly changes as the breeding season progresses (Wong-Muñoz et al. 2011), with body size either increasing, decreasing, or a mixture of both. In damselflies, adult body size is largely determined by the duration of larval development (e.g. Crowley & Johansson 2002; Strobbe & Stoks 2004), food availability (Jiménez-Cortés et al. 2012), photoperiod, or predators encountered during this stage (Teuschl et al. 2007). Typically, the environmental conditions in a given natural population are not constant throughout a season, which may alter the availability of food and nutrients (Johansson et al. 2001; Córdoba-Aguilar et al. 2010) and in turn, body size at emergence. This may be especially relevant for taxa whose adults remain on the breeding sites for long periods, such as the damselflies of the genus *Hetaerina*, which can be observed on the wing throughout the year (e.g. *H. americana*; Córdoba-Aguilar et al. 2009) or at least during an important fraction of the year (e.g. *H. vulnerata*, April–mid-December; Ramírez-Delgado et al. 2015). *H. americana* males emerge at smaller sizes during April, and reach a peak in July–October (Córdoba-Aguilar et al. 2009), whilst *H. vulnerata* males exhibit an increase in body size as the end of the season approaches. The differences in the patterns of body size seasonal variation between these closely related species may arise from overall environmental differences in their habitats. Whilst *H. americana* can be found in low-elevation, semi-tropical habitats (850 m asl; annual temperature range in Morelos, Mexico, 24–36°C), *H. vulnerata* is more typical of high-elevation, temperate regions (2450 m asl; annual temperature range in Tlaxcala, Mexico, 12–16°C), where it also undergoes winter diapause. Large *H. vulnerata* larvae may experience high mortality rates during the winter diapause (e.g. Teuschl et al. 2007), and thus smaller adults may emerge at the onset of the breeding season. Once the breeding season begins, more benign environmental conditions could allow the larvae to continue their development throughout the season, and these may eventually emerge bearing larger body sizes (Teuschl et al. 2007). The pattern may be further reinforced by viability selection acting on smaller larvae (Blanckenhorn 2000).

Second, WP of *H. vulnerata* males may increase towards the end of the season, and further exhibit positive allometry from July for two, non-mutually exclusive reasons. First, the WP of *Hetaerina* males is generally considered a costly (Grether & Grey 1996), condition-dependent sexual trait (Álvarez et al. 2013) which relates to immune function and also to food availability during the development of larvae (e.g. Contreras-Garduño et al. 2008; Outomuro et al. 2014; but see González-Santoyo et al. 2010; González-Tokman & Córdoba-Aguilar 2010; Jiménez-Cortés & Córdoba-Aguilar 2013). It appears that there is no resource allocation compromise in WP, body size, and immune function in males (Jiménez-Cortés & Córdoba-Aguilar 2013), so that by the time the males emerge, they may still invest in the development of exaggerated WP patterns, independently of body size (Jiménez-Cortés & Córdoba-Aguilar 2013). According to the “display hypothesis” (Gould 1974; Petrie 1988, 1992), only males in overall good condition will be able to invest relatively more on sexual traits, with respect to body size. As such, WP would convey certain information on the male’s “quality” to rivals. However, this explanation alone may not suffice to explain the positively allometric trend exhibited by WP in *H. vulnerata* (Eberhard et al. 2018). Second, WP of *H. vulnerata* males may exhibit positive allometry because it has evolved as a threat signal. By definition, a “pure” threat signal is used by males to intimidate rivals as to induce them to abandon a contest over a given resource (Eberhard et al. 2018). Selection on the signaller may favour dishonest signals in order to increase their perceived size and power, which may persuade similar sized rivals not to engage in a physical contest (Eberhard et al. 2018). Under this scenario, male WP in *H. vulnerata* may not be a particularly costly trait to produce. In the closely related *H. americana*, male WP has evolved through male-male competition over mating territories (Grether 1996a): males with larger amounts of WP are more successful at holding territories (Grether 1996a), and as a consequence, secure a higher number of copulations than non-territorial males (Grether 1996a; Serrano-Meneses et al. 2007). This was also true for males with experimentally enlarged WP spots (Grether 1996a). In *H. vulnerata*, male mating success is also greatly determined by the ownership of a territory (Alcock 1982; Ramírez-Delgado et al. 2015). However, WP does not appear to determine territory ownership (Ramírez-Delgado et al. 2015). In fact, territorial and non-territorial males exhibit similar amounts of WP, and also similar positive allometric slopes of WP (Álvarez et al. 2013; Ramírez-Delgado et al. 2015). Given that territorial males can be larger than non-territorial males (Álvarez et al. 2013), the latter may dishonestly produce larger-than-average WP spots in order to deceive territorial males before contests over territories take place.

Apart from variation in body size and WP, seasonal changes in adult sex ratio (Forsgren et al. 2004), sperm depletion (Preston et al. 2001), endurance rivalry (Lidgard et al. 2005), and the economic defendability of mates and/or necessary resources for reproduction (Quinn et al. 1996), are likely to shape seasonal variation in the opportunity for sexual selection (Reichard et al. 2008). Under this scenario, WP may be more relevant for male mating success during the second half of the reproductive season of *H. vulnerata*, when male density appears to be higher. Although we did not collect data on male density throughout the reproductive season, sampling effort was constant, thus sample sizes suggest an increase in the number of males at the study site. From July, a higher number of males, with larger body sizes, may compete strongly over the possession of territories, leading to an increase in overall WP, and in the allometry of WP. Nonetheless, more studies including data on male density are needed to formally test this idea.

To summarise, by emphasising the function of male sexual traits in Odonata (following Eberhard et al. 2018), it is possible to understand why WP exhibits different allometric slopes across taxa. Positive allometry should be expected for taxa bearing threat signals (e.g. *Hetaerina*), whilst isometry or negative allometry should be more common in taxa bearing courtship signals (e.g. *Calopteryx*). The study of Outomuro et al. (2014) suggests that this may be true in Odonata. In their study, Outomuro et al. (2014) found positive allometry in nine out of 12 significant MA regressions in *Hetaerina* taxa, and also positive allometry in four (*Calopteryx aequabilis, C. splendens, C. xanthostoma* and *Mnesarete pudica*) out of nine taxa in which WP has a role as both a threat and a courtship signal (isometric WP was found in *C. haemorrhoidalis, C. maculata, C. virgo, Neurobasis chinensis* and *Sapho bicolor*). Hardersen (2010), however, found what appears to be isometry and negative allometry in the WP of *C. splendens* – although the analytical method employed in the study is known to consistently underestimate allometric slopes. We believe that a macro-evolutionary comparative study, sampling a large number of odonate taxa in which the role of WP is known, and data collected considering the seasonal variation in WP shown here, would shed light on the forces shaping the allometry of WP in Odonata.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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REFERENCES

Alcock J. 1982. Post-copulatory mate guarding by males of the damselfly *Hetaerina vulnerata* Selys (Odonata: Calopterygidae). Anim Behav. 30:99–107.

Alcock J. 1987. The effects of experimental manipulation of resources on the behavior of two calopterygid damselflies that exhibit resource-defense polygyny. Can J Zool. 65:2475–2482.

Álvarez HA, Serrano-Meneses MA, Reyes-Márquez I, Jiménez-Cortés JG, Córdoba-Aguilar A. 2013. Allometry of a sexual trait in relation to diet experience and alternative mating tactics in two rubyspot damselflies (Calopterygidae: *Hetaerina*). Biol J Linn Soc. 108:521–533.

Bello-Bedoy R, González-Santoyo I, Serrano-Meneses MA, Vrech D, Rivas M, Munguía-Steyer R, Córdoba-Aguilar A. 2015. Is allometry of sexual traits adaptive? A field test with territorial damselflies. Bio J Linn Soc. 114:327–334.

Blanckenhorn WU. 2000. The evolution of body size: what keeps organisms small? Q Rev Biol. 75:385–407.

Bonduriansky R. 2007. Sexual selection and allometry: a critical reappraisal of the evidence and ideas. Evolution. 61:838–849.

Bonduriansky R, Day T. 2003. The evolution of static allometry in sexually selected traits. Evolution. 57:2450–2458.

Contreras-Garduño J, Buzatto BA, Abundis L, Nájera-Cordero K, Córdoba-Aguilar A. 2007. Wing colour properties do not reflect male condition in the American rubyspot (*Hetaerina americana*). Ethology. 113:944–952.

Contreras-Garduño J, Buzatto BA, Serrano-Meneses MA, Nájera-Cordero K, Córdoba-Aguilar A. 2008. The size of the red wing spot of the American rubyspot as a heightened condition-dependent ornament. Behav Ecol. 19:724–732.

Contreras-Garduño J, Córdoba-Aguilar A, Martínez-Becerril RI. 2011. The relationship between male wing pigmentation and condition in *Erythrodiplax funerea* (Hagen) (Anisoptera: Libellulidae). Odonatologica. 40:89–94.

Corbet P. 1999. Dragonflies: behaviour and ecology of Odonata. Revised ed. Colchester (UK): Harley Books.

Córdoba-Aguilar A. 1994. Male substrate use in relation to age and size in *Hetaerina cruentata* (Rambur) (Zygoptera: Calopterygidae). Odonatologica. 23:399–403.

Córdoba-Aguilar A, Cordero-Rivera A. 2005. Evolution and ecology of Calopterygidae (Zygoptera: Odonata): status of knowledge and research perspectives. Neotrop Entomol. 34:861–879.

Córdoba-Aguilar A, Jiménez-Cortés JG, Lanz-Mendoza H. 2009. Seasonal variation in ornament expression, body size, energetic reserves, immune response and survival in males of a territorial insect. Ecol Entomol. 34:228–239.

Córdoba-Aguilar A, López-Valenzuela A, Brunel O. 2010. Allometry in damselfly ornamental and genital traits: solving some pitfalls of allometry and sexual selection. Genetica. 138:1141–1146.

Córdoba-Aguilar A, Méndez V. 2006. Immune melanization ability and male territorial status in *Erythemis vesiculosa* (Fabricius) (Anisoptera: Libellulidae). Odonatologica. 35:193–197.

Cotton S, Fowler K, Pomiankowski A. 2004. Condition dependence of sexual ornament size and variation in the stalked-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). Evolution. 58:1038–1046.

Crowley PH, Johansson F. 2002. Sexual dimorphism in Odonata: age, size and sex ratio at emergence. Oikos. 96:364–378.

Eberhard WG. 2009. Static allometry and animal genitalia. Evolution. 63:48–66.

Eberhard WG, Rodríguez RL, Huber BA, Speck B, Miller H, Buzatto BA, Machado G. 2018. Sexual selection and static allometry: the importance of function. Q Rev Biol. 93:207–250.

Fairbairn DJ. 1997. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. Annu Rev Ecol Syst. 28:659–687.

Forsgren E, Amundsen T, Borg ÅA, Bjelvenmark J. 2004. Unusually dynamic sex roles in a fish. Nature. 429:551–554.

González-Santoyo I, Córdoba-Aguilar A, González-Tokman DM, Lanz-Mendoza H. 2010. Phenoloxidase activity and melanization do not always covary with sexual trait expression in *Hetaerina* damselflies (Insecta: Calopterygidae). Behaviour. 147:1285–1307.

González-Tokman DM, Córdoba-Aguilar A. 2010. Survival after experimental manipulation in the territorial damselfly *Hetaerina titia* (Odonata: Calopterygidae): more ornamented males are not more pathogen resistant. J Ethol. 28:29–33.

Gould SJ. 1974. The origin and function of 'bizarre' structures: antler size and skull size in the "Irish elk", *Megaloceros giganteus*. Evolution. 28:191–220.

Green AJ. 1992. Positive allometry is likely with mate choice, competitive display and other functions. Anim Behav. 43:170–172.

Grether GF. 1996a. Intrasexual competition alone favors a sexually dimorphic ornament in the rubyspot damselfly *Hetaerina americana*. Evolution. 50:1949–1957.

Grether GF. 1996b. Sexual selection and survival selection on wing coloration and body size in the rubyspot damselfly *Hetaerina americana*. Evolution. 50:1939–1948.

Grether GF, Grey RM. 1996. Novel cost of a sexually selected trait in the rubyspot damselfly *Hetaerina americana*: conspicuousness to prey. Behav Ecol. 7:465–473.

Hardersen S. 2010. Seasonal variation of wing spot allometry in *Calopteryx splendens* (Odonata Calopterygidae). Ethol Ecol Evol. 22:365–373.

Harvey PH, Pagel MD. 1991. The comparative method in evolutionary biology. Oxford (UK): Oxford University Press.

Huxley JS, Teissier G. 1936. Terminology of relative growth. Nature. 137:780–781.

Jiménez-Cortés JG, Córdoba-Aguilar A. 2013. Condition dependence and trade-offs of sexual versus non-sexual traits in an insect. J Ethol. 31:275–284.

Jiménez-Cortés JG, Serrano-Meneses MA, Córdoba-Aguilar A. 2012. The effects of food shortage during larval development on adult body size, body mass, physiology and developmental time in a tropical damselfly. J Insect Physiol. 58:318–326.

Johansson F, Stoks R, Rowe L, De Block M. 2001. Life history plasticity in a damselfly: effects of combined time and biotic constraints. Ecology. 82:1857–1869.

Kodric-Brown A, Sibly RM, Brown JH. 2006. The allometry of ornaments and weapons. Proc Natl Acad Sci USA. 103:8733–8738.

LaBarbera M. 1989. Analyzing body size as a factor in ecology and evolution. Annu Rev Ecol Syst. 20:97–117.

Lidgard DC, Boness DJ, Bowen WD, McMillan JI. 2005. State-dependent male mating tactics in the grey seal: the importance of body size. Behav Ecol. 16:541–549.

Marks LE. 1974. Sensory processes: the new psychophysics. New York (NY): Academic Press.

McArdle BH. 1988. The structural relationship: regression in biology. Can J Zool. 66:2329–2339.

McCullough EL, Ledger KJ, O'Brien DM, Emlen DJ. 2015. Variation in the allometry of exaggerated rhinoceros beetle horns. Anim Behav. 109:133–140.

Miller RG Jr. 1986. Beyond ANOVA, basics of applied statistics. New York (NY): John Wiley & Sons.

Moore AJ. 1990. The evolution of sexual dimorphism by sexual selection: the separate effects of intrasexual selection and intersexual selection. Evolution. 44:315–331.

Outomuro D, Cordero-Rivera A, Nava-Bolaños A, Córdoba-Aguilar A. 2014. Does allometry of a sexually selected ornamental trait vary with sexual selection intensity? A multi-species test in damselflies. Ecol Entomol. 39:399–403.

Petrie M. 1988. Intraspecific variation in structures that display competitive ability: large animals invest relatively more. Anim Behav. 36:1174–1179.

Petrie M. 1992. Are all secondary sexual display structures positively allometric and, if so, why? Anim Behav. 43:173–175.

Plaistow SJ, Tsuchida K, Tsubaki Y, Setsuda K. 2005. The effect of a seasonal time constraint on the development time, body size, condition, and morph determination in the horned beetle *Allomyrina dichotoma* L. (Coleoptera: Scarabaeidae). Ecol Entomol. 30:692–699.

Preston BT, Stevenson IR, Pemberton JM, Wilson K. 2001. Dominant rams lose out by sperm depletion. Nature. 409:681–682.

Quinn TP, Adkison MD, Ward MB. 1996. Behavioral tactics of male sockeye salmon (*Oncorhynchus nerka*) under varying operational sex ratios. Ethology. 102:304–322.

R Core Team. 2018. R: A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. Available from: https://www.R-project.org/ [Accessed 3 Aug 2018].

Ramírez-Delgado J, López-García K, Lara C, Serrano-Meneses MA. 2015. Wing pigmentation in males of a territorial damselfly: alternative reproductive tactics, allometry and mating success. J Insect Behav. 28:569–581.

Reichard M, Smith C, Bryja J. 2008. Seasonal change in the opportunity for sexual selection. Mol Ecol. 17:642–651.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH image to imageJ: 25 years of image analysis. Nat Methods. 9:671–675.

Serrano-Meneses MA, Córdoba-Aguilar A, Méndez V, Layen SJ, Székely T. 2007. Sexual size dimorphism in the American rubyspot: male body size predicts male competition and mating success. Anim Behav. 73:987–997.

Sharma MD, Tregenza T, Hosken DJ. 2011. Sex combs, allometry, and asymmetry in *Drosophila*. Biol J Linn Soc. 103:923–934.

Simmons LW, Tomkins JL. 1996. Sexual selection and the allometry of earwig forceps. Evol Ecol. 10:97–104.

Sokal RR, Rohlf FJ. 2003. Biometry: the principles and practice of statistics in biological research, 3rd ed. San Francisco (CA): W.H. Freeman and Company.

Strobbe F, Stoks R. 2004. Life history reaction norms to time constraints in a damselfly: differential effects on size and mass. Biol J Linn Soc. 83:187–196.

Suhonen J, Rantala MJ, Honkavaara J. 2008. Territoriality in odonates. In: Córdoba-Aguilar A, editor. Dragonflies and damselflies: model organisms for ecological and evolutionary research. Oxford (UK): Oxford University Press; p. 203–218.

Teuschl Y, Reim C, Blanckenhorn WU. 2007. Correlated responses to artificial body size selection in growth, development, phenotypic plasticity and juvenile viability in yellow dung flies. J Evol Biol. 20:87–103.

Tomkins JL, Kotiaho JS, LeBas NR. 2005. Matters of scale: positive allometry and the evolution of male dimorphisms. Am Nat. 165:389–402.

Warton DI. 2007. Robustness to failure of assumptions of tests for a common slope amongst several allometric lines – a simulation study. Biom J. 49:286–299.

Warton DI, Duursma RA, Falster DS, Taskinen S. 2012. smatr 3 — an R package for estimation and inference about allometric lines. Methods Ecol Evol. 3:257–259.

Warton DI, Wright IJ, Falster DS, Westoby M. 2006. Bivariate line-fitting methods for allometry. Biol Rev. 81:259–291.

West-Eberhard MJ. 2014. Darwin's forgotten idea: the social essence of sexual selection. Neurosci Biobehav Rev. 46:501–508.

Wilkinson GS, Dodson GN. 1997. Function and evolution of antlers and eye stalks in flies. In: Choe JC, Crespi BJ, editors. The evolution of mating systems in insects and arachnids. Cambridge (UK): Cambridge University Press; p. 310–328.

Wolak ME, Fairbairn DJ, Paulsen YR. 2012. Guidelines for estimating repeatability. Methods Ecol Evol. 3:129–137.

Wong-Muñoz J, Córdoba-Aguilar A, Cueva del Castillo R, Serrano-Meneses MA, Payne J. 2011. Seasonal changes in body size, sexual size dimorphism and sex ratio in relation to mating system in an adult odonate community. Evol Ecol. 25:59–75.

Worthington AM, Berns CM, Swallow JG. 2012. Size matters, but so does shape: quantifying complex shape changes in a sexually selected trait in stalk-eyed flies (Diptera: Diopsidae). Biol J Linn Soc. 106:104–113.

El caballito escarlata de montaña y la coloración animal

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El caballito escarlata de montaña y la coloración animal

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Resumen.

El origen del color en los animales ha sido un tema de intensa investigación desde el inicio de la biología. Su estudio nos ha permitido comprender los mecanismos detrás de tan colorido espectáculo natural: los pigmentos orgánicos y las microestructuras iridiscentes. Sin embargo, las hipótesis evolutivas que expliquen el mantenimiento de tan llamativos caracteres continúan siendo debatidas. A manera de revisión, el presente artículo explora las posibles causas de la coloración animal, utilizando como ejemplo a un insecto distribuido en el centro de México: el caballito escarlata de montaña (*Hetaerina vulnerata* Hagen in Selys, 1853).

Palabras clave: Coloración animal, Odonata, selección sexual.

Abstract.

The origin of animal coloration has been a subject of intense research since the beginning of biological studies. Its examination has allowed us to understand the mechanisms behind this colorful natural spectacle: organic pigments and iridescent microstructures. However, the evolutionary hypotheses that explain the maintenance of such striking characters, continue to be debated. By way of revision, this article explores the possible causes of animal coloration, using as an example an insect distributed in central Mexico: the canyon rubyspot (*Hetaerina vulnerata* Hagen in Selys, 1853).

Key words: Animal coloration, Odonata, sexual selection.

Introducción.

Volando sobre un arroyo a las afueras de San Tadeo Huiloapan en el estado de Tlaxcala, los caballitos escarlatas de montaña presumen su característico color rojo cobrizo en la base de las alas. Un color notable entre otras libélulas y caballitos del diablo de la zona, que a los ojos de cualquier entomólogo los delatan como individuos de la especie *Hetaerina vulnerata*. Aún más, si dicho científico posee una mirada crítica, naturalmente se preguntará: ¿por qué este insecto presenta semejante brillo escarlata? Esta apasionante búsqueda por una explicación al origen de la coloración animal no sólo ha sido explorada por grandes naturalistas como Charles Darwin y Alfred Russel Wallace, sino que también ha sido examinada por disciplinas tan complejas como lo son la fisiología, la genética e incluso la física. A pesar de que su estudio nos ha permitido entender muchos procesos a los cuales están sometidos los organismos en la naturaleza, hasta hoy no se ha encontrado una respuesta definitiva a semejante espectáculo.

Figura 1. Río en las afueras de San Tadeo Huiloapan, Tlaxcala / Autor: M.A. Serrano-Meneses.

Si uno observa atentamente a los animales en el mundo natural, se dará cuenta que en la naturaleza se presenta una amplia gama de colores, desde el rojo intenso en el plumaje de una guacamaya, hasta la garganta violeta de un colibrí. Desde el verde pálido de algunos saltamontes hasta el azul iridiscente de la mariposa *Morpho menelaus*. No obstante, si afinamos la mirada podríamos suponer que no todos los colores se generan de la misma manera y que cada plumaje, escama o pelaje pintoresco ha de tener un origen particular. Los colores en los organismos vivos se pueden formar por dos mecanismos distintos, por la presencia de pigmentos químicos o por sofisticadas microestructuras que absorben, modifican y reflejan longitudes de onda de luz específicas. Las múltiples combinaciones entre estos mecanismos son la receta secreta de animales como el camaleón o el pulpo mimo, para generar los complicados patrones de coloración que los hacen maestros del camuflaje (Maynard-Smith & Harper, 1995).

¿A qué se debe el color?

Los animales pueden sintetizar pigmentos dentro de su cuerpo o adquirirlos mediante el consumo de alimentos que contengan una variedad de ellos. El pigmento más común en el planeta es la melanina, un polímero sintetizado a partir de la tirosina, que en diferentes concentraciones puede producir toda una gama de grises: desde el cabello más oscuro hasta los ojos más avellanados. Incluso se ha observado que los rojos brillantes en las alas de algunas especies de caballitos del diablo, se deben a tan abundante pigmento. Todos los organismos llevan a cabo la melanogénesis dentro de sí, es decir, que producen dicho pigmento en células especializadas sin necesidad de ingerirlo. En cambio, otros tipos de pigmento deben ser adquiridos mediante la dieta. Ese es el caso de los carotenoides, famosas moléculas responsables de pintar de rosa a los inconfundibles flamencos. Estos pigmentos orgánicos, que se encuentran en plantas y hongos, tienen la capacidad de generar variaciones del rojo, naranja y amarillo. Actualmente se conoce una vasta cantidad de pigmentos distintos: las pterinas que modifican la coloración de los ojos de las moscas, las psitacofulvinas que colorean a los loros, las porfirinas que, mediante la ayuda de átomos de cobre en su estructura, bañan de un verde vívido a los quetzales y la biliverdina que, de manera similar al pigmento anterior, produce el característico color azul del cascarón de los huevos de algunas aves (Caro et al., 2017) (Fig. 2).

Figura 2. Caballito Multicolor (*Hesperagrion heterodoxum*) de San Tadeo Huiloapan. Se puede apreciar el azul formados por tetrapirroles, el rojo por los carotenoides, y el anaranjado por omocromos a lo largo del cuerpo. Autor: M.A. Serrano-Meneses.

Por otro lado, se encuentra la coloración estructural que, como su nombre lo indica, depende de microestructuras en los tejidos de los organismos que dispersan la luz que llegan a ellas. Esta característica, no sólo es capaz de producir azules o violetas intensos, sino que además puede generar cambios de color dependiendo del ángulo en que incida la luz. A este reflejo se le conoce como iridiscencia o de tipo espejo, y es el responsable de regalarnos tan elegantes plumajes como el del cuello del pavo real o el brillante verde de algunas avispas y escarabajos (Caro et al., 2017) (Fig. 3).

Ahora, si bien conocemos las moléculas y estructuras que dan origen a tan alta diversidad de colores en el mundo animal, aún no hemos resuelto por qué, si todas las libélulas y caballitos del diablo de San Tadeo Huiloapan, comparten el mismo arroyo, sólo las *Hetaerina vulnerata* presentan semejante pigmentación alar.

Figura 3. Abeja de la orquídea (*Euglosa obrima*). Se observa coloración corporal verde metálica, debido a los arreglos microestructurales en combinación de pigmentos. Autores: O. García-Miranda y A.I. Carrillo-Muñoz.

Función y evolución del color.

Como toda característica fisiológica y morfológica en los animales, la coloración se encuentra bajo selección natural y sexual. En cada especie, la coloración tiene una función distinta que, si bien a veces pasa desapercibida, juega un papel crítico en su supervivencia. Un buen ejemplo del uso del color es el del camuflaje, que se define como el cambio de color corporal que conllevan algunos organismos para igualarse a su entorno y pasar inadvertidos ante posibles presas o depredadores (como lo sucedido en la polilla *Biston betularia*, ver Cuadro 1).

Cuadro 1. Melanismo industrial: un ejemplo de la evolución del color corporal (Kettlewell 1955).

Biston betularia, es una especie de polilla en la que existen dos tipos de coloración: las blancas con puntos negros y las totalmente negras. El comportamiento habitual de ambos tipos es el de descansar sobre los abedules, camuflándose entre las cortezas y los líquenes de su tronco. Viviendo en los bosques ingleses del norte, se mantenía una población estable hasta que, con la llegada de la revolución industrial, en la zona de Manchester, un exceso de expulsión de carbón en la atmósfera ennegreció a los troncos aledaños a las fábricas y todo cambió. En esa época se observó un cambio en el número de individuos de cada color, de ser las blancas con puntos negros las más comunes, con el pasar de los años este tipo de polilla fue disminuyendo, generando que por el contrario las negras dominaran los troncos cercanos a las fábricas. Para explicar este cambio en la densidad poblacional de *B. betularia* se buscaron los mecanismos de selección natural que estuvieran ejerciendo esta presión. En los troncos que una vez fueron claros y con líquenes, las polillas blancas podían pasar desapercibidas para sus depredadores con mayor facilidad que las polillas oscuras, pero cuando lo troncos de los árboles se ennegrecieron debido a la contaminación industrial, fueron las polillas oscuras las que tuvieron ventajas en pasar desapercibidas ante los depredadores. Estas presiones selectivas generan cambios en las frecuencias genéticas, haciendo que las formas oscuras fueran más frecuentes en las poblaciones de esta famosa polilla. Actualmente, el caso de la polilla de los abedules es utilizado como uno de los más famosos ejemplos de la evolución de la coloración animal (Fig. 4).

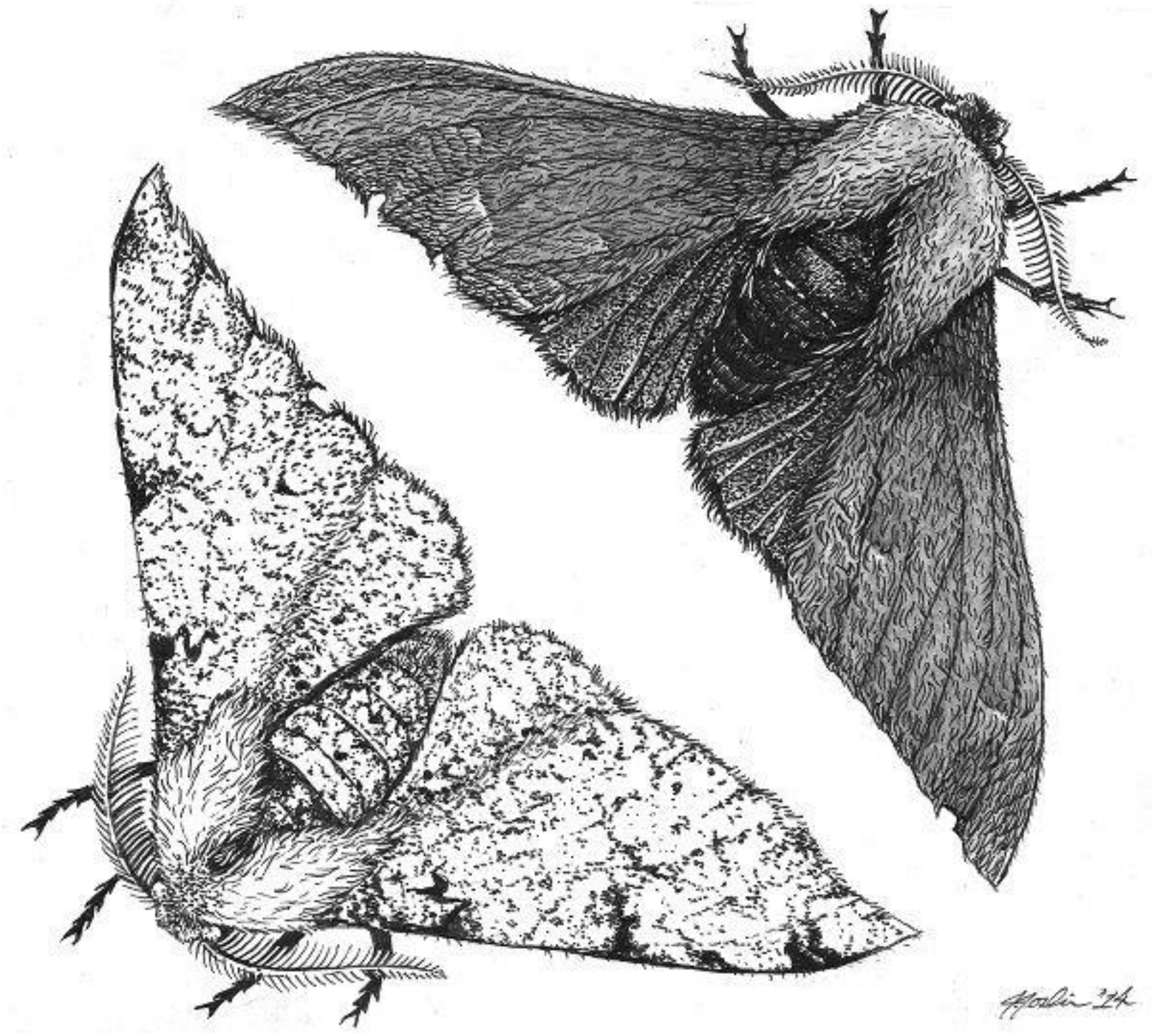

Figura 4. Polillas de los abedules (*Biston betularia*). Ambos tipos de coloración, blanca con puntos negros y totalmente negra. Autora: J. Joslin, 2014. Grafito y tinta sobre papel.

Usualmente el color corporal es utilizado como medio de comunicación intra- e interespecífico. Estas señales pueden ser usadas en diferentes contextos, para evitar ser comido por un depredador, para la adquisición de algún alimento, para evadir ciertos parásitos, para incrementar la termorregulación o para promover la reproducción. Por ejemplo, en el caso de la elección de pareja, algunos colores brillantes de los machos sirven para reflejar la calidad de sus portadores. El color también puede señalizar el estatus social de los machos, no sólo ante las hembras, sino también ante otros posibles competidores del mismo sexo (Bradbury & Vehrencamp, 2011). Como en algunas libélulas macho que, entre más brillante sea el color de sus alas, mayor será su sistema inmune. Esta coloración se muestra mediante despliegues elaborados que son evaluados por las posibles parejas y competidores. La elección femenina recae de esta manera, en escoger al macho que mejor demuestre su calidad ante los demás. Sin embargo, estos rasgos a veces pueden ser engañosos. Existen machos que engañan a las hembras, brillan mucho, pero tienen una baja calidad genética. También hay los que engañan a sus competidores, imitando la coloración y el comportamiento femenino para evitar peleas y conseguir mayores cópulas (Arnqvist & Rowe, 2005).

Caso de estudio: Odonata.

De este modo, regresamos al orden Odonata, un grupo animal perteneciente a la Clase Insecta, conformado por las libélulas y los caballitos del diablo. Actualmente se reconoce la existencia de 5,200 especies vivas y 800 especies extintas de odonatos. A pesar de que su ciclo de vida es sumamente interesante, con la mayoría de las larvas con hábitos acuáticos, y con los adultos atraídos fuertemente a cuerpos de agua para encontrar parejas potenciales y reproducirse, esta no es la razón precisa de su importancia como organismo modelo. A continuación, se enumeran algunas características fisiológicas, conductuales y ecológicas, que hacen de los odonatos un grupo atractivo para el estudio de la evolución del color, tanto corporal como alar (Corbet, 1999) (Fig. 5).

Figura 5. Libélula Anillada de Frente Amarilla (*Cordulegaster diadema*) de San Tadeo Huiloapan. Se observan los anillos negros formados por melanina, intercalados de los amarillos formados de la acumulación de pterinas. Autor: M.A. Serrano-Meneses.

i) La pigmentación alar, a diferencia de la coloración corporal, se fija una vez que el organismo alcanza la edad reproductiva y generalmente son los machos los que presentan una mayor expresión de este rasgo, de manera que este carácter se expresa diferencialmente entre los sexos.

ii) Los odonatos adultos poseen tamaños, coloraciones y comportamientos visibles ante sus depredadores y presas, pero también ante los investigadores (Fig. 6).

iii) Por último, muchas especies de odonatos, al desarrollar solo una o dos generaciones de individuos al año y al poder ser marcados individualmente sin interferir en sus actividades, pueden ser monitoreados exitosamente y sin demasiado esfuerzo.

Figura 6. Coloración de las alas de algunos machos del orden Odonata. De izquierda a derecha: *Calopteryx maculata*, *Libellula pulchella* y *Neurobasis chinensis.* Autor: M.A. Serrano-Meneses.

Estos atributos han generado que varias especies, no sólo sean utilizadas como indicadores de la calidad de los ambientes acuáticos, sino que además han ayudado a biólogos evolutivos alrededor del mundo a proponer cinco hipótesis sobre la evolución y el mantenimiento de la coloración animal.

Hipótesis del origen de la coloración.

Si los caracteres de los organismos machos o hembras son adaptaciones específicas al ambiente en donde se distribuyen y al uso que le dan a dicho hábitat, entonces es lógico pensar que la pigmentación será particular a dicho sitio. Si ambos sexos ocupan un nicho distinto, aunque ocupen la misma área, también se espera que presenten diferencias en sus coloraciones. A esta proposición, se le conoce como la hipótesis de la utilización diferencial de nicho.

La hipótesis de advertencia contra depredadores, por otro lado, sugiere que la pigmentación ha evolucionado para comunicar que los organismos portadores de estos patrones vistosos son presas de difícil adquisición. Los depredadores entonces percibirán en ellos un gasto innecesario, ya que dicho color puede significar un grado de toxicidad elevado, lo que haría de estos odonatos pigmentados no aptos para su consumo. Esta hipótesis no se ha podido demostrar del todo en estos insectos, sin embargo, a principios de siglo, dos investigadores suecos, encontraron que el tamaño y la intensidad de la pigmentación alar de dos especies diferentes del género *Calopteryx* están bajo presiones selectivas distintas. En una especie se favorece el aumento del tamaño de la mancha alar y en la otra su disminución. Lo impactante del caso es que en ambas especies la depredación por las aves es la que modifica el tamaño de sus pigmentos alares (Svensson & Friberg, 2007).

En los caballitos del diablo del género *Mnais*, originarias del Japón, existen dos especies que a veces comparten la misma área de reproducción. Cuando esto ocurre, una de ellas, *Mnais pruinosa*, presenta un tipo de macho menos pigmentado que la otra, *Mnais costalis*. Este caso ejemplifica la hipótesis del desplazamiento ecológico de caracteres, que sugiere que la evolución del pigmento se moldea para prevenir los apareamientos interespecíficos que disminuirían la adecuación de las poblaciones. En otras palabras, esta hipótesis propone que la coloración alar se ha moldeado para el reconocimiento específico cuando existen más de una especie en el mismo sitio (Tsubaki & Okuyama, 2015).

Si bien se ha explicado hasta ahora las posibles hipótesis del origen de la pigmentación utilizando a los odonatos como modelo, se ha omitido un comportamiento fundamental para comprender las últimas hipótesis. En los odonatos existen especies territoriales y no territoriales. Es importante mencionar de esta manera, que todas las hipótesis anteriormente propuestas pueden aplicarse mayormente a las especies que realizan búsquedas activas de parejas potenciales, es decir, a las no territoriales. Ya que éstas no defienden un área en particular para conseguir una cópula exitosa y actualmente se desconoce el papel que juega la pigmentación alar en su reproducción. No obstante, el origen del color de las alas en estos odonatos no territoriales es posiblemente explicado por su nicho ecológico, o como una advertencia contra depredadores.

En algunas especies territoriales, como *Plathemis lydia*, los machos reconocen el estatus social de sus coespecíficos, lo que permite que las interacciones agresivas disminuyan y que más de un macho permanezca en un solo territorio. Este comportamiento concuerda con lo propuesto por la hipótesis del estatus social, que sugiere que el desarrollo de este rasgo es utilizado para evitar conflictos innecesarios que puedan ser bastantes costosos (Wolf & Campanella, 1974) (Suhonen et al., 2008).

Finalmente existe una última explicación al problema del origen del pigmento alar en los animales, y es probable que sea la más adecuada para comprender por fin el mantenimiento del color escarlata en *Hetaerina vulnerata*. El caballito escarlata de montaña, como en otras especies, hace uso de sus patrones de pigmentación alar durante el cortejo sexual y durante las interacciones agonísticas con otros machos. Debido a este comportamiento, en varias especies de Odonata, se ha demostrado que la pigmentación alar de los machos está sometida a selección sexual, concediendo un mayor éxito reproductivo a los portadores que exageren su expresión (Serrano-Meneses et al., 2017) (Fig. 7).

Se ha observado que la pigmentación alar en *Hetaerina vulnerata* se relaciona con su éxito reproductivo, ya que para los machos que invierten más en este rasgo es más probable encontrar parejas potenciales y realizar una defensa de su territorio de una manera más eficiente. Dicho pigmento está ligado a la condición energética de su portador (su potencial para mantener un territorio) y/o a su calidad inmune (capacidad contra patógenos). Este caballito del diablo de Tlaxcala es un ejemplo claro de lo propuesto por la última hipótesis: la selección sexual. Esta hipótesis sugiere que la variación de este tipo de rasgos es moldeada principalmente mediante competencia intraespecífica (Andersson, 1994; Grether, 1997).

Figura 7. Caballito escarlata de montaña (*Hetaerina vulnerata*) macho. Autor: M.A. Serrano-Meneses.

Conclusión.

A pesar de que el origen de la pigmentación alar es particular para cada especie, hasta ahora se han desarrollado numerosas hipótesis acerca de la evolución de este rasgo. Cada año se suman más investigadores a esta apasionante búsqueda, trabajando incansablemente sobre hombros de gigantes. Desde esa perspectiva, hoy comprendemos algunas posibles causas de tan particular brillo escarlata, y nos recuerda que incluso en un arroyo a las afueras de un pueblo tlaxcalteca, la respuesta a tan inmensa pregunta se encuentra al alcance de todos nosotros. Para aprehenderla, sólo se necesita una mirada crítica y unos cuantos caballitos del diablo.

Referencias

Andersson, M. (1994). *Sexual selection* (Vol. 72). Princeton, NJ: Princeton University Press.

Arnqvist, G. & Rowe, L. (2005). *Sexual Conflict*. Princeton: Princeton University Press.

Bradbury, J. W. & Vehrencamp, S. L. (2011). *Principles of animal communication* (2nd ed.). Sunderland: Sinauer Associates, Inc., Publishers.

Caro, T., Caswell Stoddard, M. & Stuart-Fox, D. (2017). Animal coloration: production, perception, function and application. *Philosophical Transitions of the Royal Society, B*, *372*(1724). doi: https://doi.org/10.1098/rstb.2017.0047

Corbet, P. S. (1999). *Dragonflies: behaviour and ecology of Odonata*. Colchester: Harley Books.

Grether, G. F. (1997). Survival cost of an intrasexually selected ornament in a damselfly. *Proceedings of the Royal Society of London, B*, *264*(1379), 207–210. doi: 10.1098/rspb.1997.0029

Maynard-Smith, J. & Harper, D. (1995). Animal Signals: Models and Terminology. *Journal of Theoretical Biology*, *177*(3), 305–311. doi: 10.1006/jtbi.1995.0248

Serrano-Meneses, M. A., Córdoba-Aguilar, A., Méndez, V., Layen, S. J. & Székely, T. (2007). Sexual size dimorphism in the American rubyspot: male body size predicts male competition and mating success. *Animal Behaviour*, *73*(6), 987–997. doi: 10.1016/j.anbehav.2006.08.012

Suhonen, J., Rantala, M. J. & Honkavaara, J. (2008). Territoriality in odonates. *Dragonflies and damselflies: model organisms for ecological and evolutionary research*. Oxford: Oxford University Press. 203-217.

Svensson, E. I. & Friberg, M. (2007). Selective Predation on Wing Morphology in Sympatric Damselflies. *The American Naturalist*, *170*(1), 101–112. doi: 10.1086/518181

Tsubaki, Y. & Okuyama, H. (2015). Adaptive loss of color polymorphism and character displacements in sympatric Mnais damselflies. *Evolutionary Ecology*, *30*(5), 811–824. doi: 10.1007/s10682-015-9778-3

Wolf, L. L. & Campanella, P. J. (1974). Temporal Leks as a Mating System in a Temperate Zone Dragonfly (Odonata: Anisoptera) I: Plathemis Lydia (Drury). *Behaviour*, *51*(1-2), 49–87. doi: 10.1163/156853974x00147

New records from the Chimalapas-Uxpanapa region, Mexico (Odonata: Calopterygidae, Heteragrionidae, Polythoridae, Thaumatoneuridae, Coenagrionidae, Gomphidae, Libellulidae)

Aldo Isaac CARRILLO-MUÑOZ y Oscar GARCÍA-MIRANDA. (2020).

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New records for the Chimalapas-Uxpanapa Region, Mexico (Odonata: Calopterygidae, Heteragrionidae, Polythoridae, Thaumatoneuridae, Coenagrionidae, Gomphidae, Libellulidae)

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Abstract. During a collecting trip to five tropical rainforest sites in the Chimalapas-Uxpanapa region in an altitudinal gradient of 155–499 m a.s.l., a total of sixteen species of odonates new to the region were recorded. Poorly studied species such as *Erpetogomphus ophibolus* Calvert, 1905, *Hetaerina infect*a Calvert, 1901, *Heteragrion alienum* Williamson, 1919, and *Heteragrion tricellulare* Calvert, 1901, were recorded. *Ischnura demorsa* Hagen, 1861, and *Paraphlebia* spp. are discussed in greater detail. These records add to knowledge of odonate distribution from Oaxaca and Veracruz.

Further key words. Dragonfly, damselfly, Anisoptera, Zygoptera, conservation, Neotropical realm

Introduction

According to the most recent published revision of the biodiversity of Mexican odonate species, and the sampled-based and coverage-based rarefaction curves calculated by Cuevas-Yañez et al. (2017), there are 356 species of Odonata (205 Anisoptera and 151 Zygoptera) in Mexico, of which 137 species occur in the state of Oaxaca and 222 in Veracruz (González-Soriano & Novelo Gutiérrez 2014). Between Veracruz and Oaxaca is the Chimalapas-Uxpanapa (CU) region, a priority area for conservation in Mexico. Although it represents only 0.57 % of the country's total area, is considered to be a highly important global centre of biodiversity (Arriaga et al. 2000; Conabio 2010). This high diversity results from its altitudinal range of 100 to 2 700 m a.s.l., its intermingling ecosystems dominated by tropical rainforest (56 %), and climatic influences from both the Pacific and the Gulf of Mexico (Arriaga et al. 2000; Peterson et al. 2003). Although new species of Coleoptera and Lepidoptera have recently been described from the CU region (*e.g.*, Ariza-Marín et al. 2019; Callaghan et al. 2013), of the 250 species of Odonata present in Oaxaca and Veracruz, to date none has been recorded from the CU region, and for most species recorded from both states, overall distribution and population trends are unknown (IUCN 2019). The challenging topography of the area hinders fieldwork and may explain this lack of data (Arriaga et al. 2000; Lira-Torres & Briones-Salas 2012). This note presents preliminary data from a project aiming to describe the distribution of dragonflies and damselflies of the Mexican Neotropical zone.

Material and methods

Data were collected from 09-x- to 12-x-2018 in the municipalities of Uxpanapa, Veracruz and Santa María Chimalapa, Oaxaca, along a transect extending approximately 13 kilometres. Five sites between 155 and 499 m a.s.l. with tropical rainforest vegetation were examined. The climate of the region is warm, with an annual precipitation *ca* 1 000 mm (Arriaga et al. 2000). Sampling was between 12 pm and 4 pm (CST; UTC -6 h), always when the air temperature was greater than 18°C. Adult odonates were collected with an aerial net and preserved in 70 % ethanol. Specimens were identified under a stereoscopic microscope (Nikon DS-Fi3), with reference to the keys by Bick & Bick (1990), Garrison (1984, 1990, 1994), Garrison et al. (2006, 2010), Heckman (2008), Leonard (1977), Meurgey (2016), Paulson (2009), Peña-Olmedo (1989), Westfall (1992) and Williamson (1919). Collections were made by AICM; specimens are deposited in the Colección Entomológica of the Universidad de las Américas Puebla (UDLAP).

List of collecting sites

(**1**) Municipality of Uxpanapa, Veracruz (17°10’23.567” N, 94°28’18.289” W, 155 m a.s.l.), 09-x-2018. Slow running river next to a wooden house with scattered patches of shrubs and large-leaved plants, relatively well conserved. (**2**) Municipality of Santa María Chimalapas, Oaxaca (17°7’58.436” N, 94°27’34.455” W, 499 m a.s.l.), 11-x-2018. Fast running river with some cascades and patches of slow water stream with very large-leaved plants, relatively well conserved. (**3**) Municipality of Uxpanapa, Veracruz (17°10’37.736” N, 94°28’42.761” W, 159 m a.s.l.), 12-x-2018. Next to a shed along a rocky shore with slow running water and covered by tree shadows. (**4**) Municipality of Uxpanapa, Veracruz (17°11’49.132” N, 94°28’43.316” W, 358 m a.s.l.), 12-x-2018. Tiny slow running streams along a disturbed pathway opened with a backhoe several years ago. (**5**) Municipality of Uxpanapa, Veracruz (17°12’12.373” N, 94°28’55.246” W, 194 m a.s.l.), 12-x-2018. Slow wide turbid river in a disturbed site with cattle.

Results

Sixteen species new for the CU region are listed below. Taxa new to Oaxaca and Veracruz are not reported here. The topographic position of the localities is provided in Figure 1. Numbers in bold in parentheses correspond with the list of collecting sites.

Family Calopterygidae

1. ***Hetaerina infecta* Calvert, 1901**
   (2,3) 8♂ 11-12-x-2018.
2. ***Hetaerina occisa* Hagen, 1853**
   (1,3,4,5) 9♂1♀ 9-12-x-2018.
3. ***Hetaerina sempronia* Hagen, 1853**
   (5) 1♂ 12-x-2018.

Family Heteragrionidae

4. ***Heteragrion alienum* Williamson, 1919**
   (3,5) 10♂1♀ 12-x-2018.
5. ***Heteragrion tricellulare* Calvert, 1901**
   (2) 1♂ 11-x-2018.

Family Polythoridae

6. ***Cora marina* Selys, 1868**
   (4) 2♂ 12-x-2018.

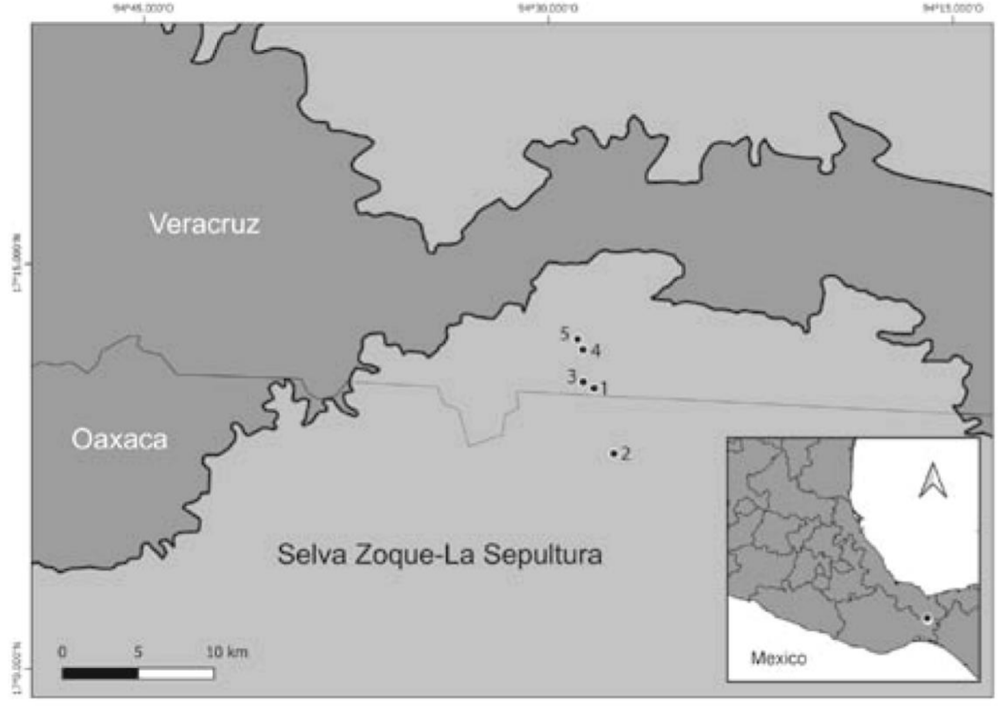

Fig. 1. Topographic position of the collecting sites of the sixteen species newly recorded for the Chimalapas-Uxpanapa (CU) region. Light grey: Selva Zoque-La Sepultura on the CU region. Detailed information of each site is stated in the main text.

Family Thaumatoneuridae

7. *Paraphlebia* spp.
(2) 1♀ 11-x-2018.

Family Coenagrionidae

8. *Acanthagrion quadratum* Selys, 1876
(1) 2♂ 9-x-2018.
9. *Argia pulla* Hagen, 1865
(1) 3♂ 9-x-2018.
10. *Argia cuprea* Hagen, 1861
(3,4) 4♂ 12-x-2018.
11. *Enallagma novaehispaniae* Calvert, 1907
(3) 1♂ 12-x-2018.
12. *Ischnura demorsa* Hagen, 1861
(1) 1♀ 9-x-2018.

Family Gomphidae

13. *Erpetogomphus ophibolus* Calvert, 1905
(4) 1♂ 12-x-2018.

Family Libellulidae

14. *Brechmorhoga vivax* Calvert, 1906
(3) 1♂ 12-x-2018.
15. *Micrathyria dictynna* Ris, 1919
(3) 1♂ 12-x-2018.
16. *Micrathyria ocellata* Martin, 1897
(3) 1♂ 12-x-2018.

Discussion

Most of the recorded odonates in this study have a widespread distribution. However, we highlight the presence of *Erpetogomphus ophibolus*, *Hetaerina infect*a, *Heteragrion alienum*, *H. tricellulare* and *Paraphlebia spp.* in the Chimalapas-Uxpanapa region, because, although they are within their restricted expected range, these records increase the scarce distributional information of these species. At sites 1, 2, 3 and 5, 38.5 % of families, 13.4 % of genera and 6.3 % of species of Odonata known from Veracruz are represented, and at site 4, 30.8 % of families, 7.1 % of genera but only the 2.8 % of the species from Oaxaca are represented.

It is important to mention that the specimen of *Ischnura demorsa* had similar coloration to the male, so we consider it an androchromatypic female (Paulson 2009). The female of *Paraphlebia spp.* could not be identified at the species level because there are no identification keys for females of this genus (R. Novelo Gutiérrez pers. comm.). Although the collection site for this specimen is within the estimated distribution range for *P. quinta* (Cuevas-Yañez et al. 2015), we decided not to record it as belonging to this species. A recent master's thesis was published in the National Autonomous University of Mexico (UNAM) digital library, which based on molecular data, describes ten new species of *Paraphlebia* in the Neotropical region (Ortega-Salas 2017). This thesis contains only an identification key for the males of this species, so it was impossible for us to determine the species of the female collected. In addition, the female possessed a pattern of thoracic coloration similar to *Paraphlebia* sp. nov. 5 as described by the same author in his taxonomic review. Therefore, with the colour pattern, coupled with the recent discovery of new *Paraphlebia* species, we suggest that the specimen collected should be considered as undetermined for now. More odonatological surveys are needed at the site in order to determine the taxonomic status of this likely new species.

Most of the odonatological records in the state of Oaxaca and Veracruz have been from places near the coast such as Santa María Huatulco, Catemaco, La Tinaja and the Reserva de la Biosfera Los Tuxtlas (Anderson & Grether 2009; Salas-Arcos 2010; Garrison & González-Soriano 1988; Novelo Gutiérrez 2009). For this reason the odonatological records reported in this note are relevant not only because they reveal the presence of these species but also because they suggest that the CU region could be a potential dispersal corridor for odonates within Neotropical Mexico (Guevara 1995).

Although the Neotropics is one of the most diverse regions in the country, there are still areas such as the CU region that, due to its complex topography, have not been well explored (Von Ellenrieder 2009). The CU region consists largely of threatened cloud forest that, due to anthropogenic pressures, is in danger of disappearing (Williams-Linera et al. 2002), thus reducing the distribution of poorly studied genera such as *Paraphlebia* (Cuevas-Yañez et al. 2015). The fact that we found an unidentified female of that genus provides strong justification for protecting this area, not only for its potential biodiversity but for its unknown ethodiversity (Cordero-Rivera 2017). Hence, it is considered that the CU region is an area in urgent need of further odonatological surveys.

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References

Anderson C.N. & Grether G.F. 2009. Interspecific aggression and character displacement of competitor recognition in Hetaerina damselflies. *Proceedings of the Royal Society B: Biological Sciences* 277: 549-555

Ariza-Marín E.R., Reyes-Castillo, P. Moctezuma V. & Sánchez-Huerta J.L. 2019. A new species of Vindex Kaup (Coleoptera: Scarabaeoidea: Passalidae: Passalinae: Proculini) from Oaxaca, Mexico. *Neotropical Entomology* 48: 121-125

Arriaga L., Espinoza J.M., Aguilar C., Martínez E., Gómez L. & Loa E. 2000. Regiones terrestres prioritarias de México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México

Bick G.H. & Bick J.C. 1990. A revision of the neotropical genus *Cora* Selys, 1853 (Zygoptera: Polythoridae). *Odonatologica* 19: 117-143

Callaghan C.J., Llorente-Bousquets J. & Luis-Martinez A. 2013. A new species of *Euselasia* Hübner from Meso-America and Mexico with notes on the *eurypus* group (Lepidoptera, Riodinidae). *Zootaxa* 3701: 54-62

CONABIO 2010. El Bosque Mesófilo de Montaña en México: Amenazas y Oportunidades para su Conservación y Manejo Sostenible. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México, D.F.

Cordero-Rivera A. 2017. Behavioral diversity (ethodiversity): a neglected level in the study of biodiversity. *Frontiers in Ecology and Evolution*: 5: 7. doi: 10.3389/fevo.2017.00007

Cuevas-Yañez K., Espinosa-Rivera J.C., Martínez-Falcón A.P. & Córdoba-Aguilar A. 2017. Are all Mexican odonate species documented? An assessment of species richness. *Systematics and Biodiversity* 15: 253-258

Cuevas-Yañez K., Rivas M., Muñoz J. & Córdoba-Aguilar A. (2015). Conservation status assessment of Paraphlebia damselflies in Mexico. *Insect Conservation and Diversity* 8: 517-524

Garrison R.W. 1984. Revision of the genus *Enallagma* of the United States west of the Rocky Mountains and identification of certain larvae by discriminant analysis (Odonata: Coenagrionidae). *University of California Publications in Entomology* 105: 1-129

Garrison R.W. 1990. A synopsis of the genus *Hetaerina* with descriptions of four new species (Odonata: Calopterygidae). *Transactions of the American Entomological Society* 116: 175-259

Garrison R.W. 1994. A revision of the New World genus *Erpetogomphus* Hagen in Selys (Odonata: Gomphidae). *Tijdschrift voor Entomologie* 137: 173-270

Garrison R.W. & González-Soriano E. 1988. Population dynamics of two sibling species of Neotropical damselflies, *Palaemnema desiderata* Selys and *P. paulitoyaca* Calvert (Odonata: Platystictidae). *Folia entomologica Mexicana* 76: 5-24

Garrison R.W., von Ellenrieder N. & Louton J.A. 2006. Dragonfly genera of the New World: an illustrated and annotated key to the Anisoptera. The Johns Hopkins University Press, Baltimore

Garrison R.W., von Ellenrieder N. & Louton J.A. 2010. Damselfly genera of the New World: an illustrated and annotated key to the Zygoptera. The Johns Hopkins University Press, Baltimore

González-Soriano E. & Novelo Gutiérrez R. 2014. Biodiversidad de Odonata en México. *Revista mexicana de Biodiversidad* 85: 243-251

Guevara S. 1995. Connectivity: key in maintaining tropical rainforest landscape diversity. In: Halladay P. & Gilmour D.A. (Eds), Conserving biodiversity outside protected areas. The role of traditional agro-ecosystems. IUCN, Gland, Switzerland, and Cambridge, UK: 63-74

Heckman C.W. 2008. Encyclopedia of South American Aquatic Insects: Odonata – Zygoptera. Springer Press

IUCN. 2019. The IUCN Red List of Threatened Species. Version 2019-3. Online on the internet, URL (26-xii-2019): http://www.iucnredlist.org

Leonard J.W. 1977. A revisionary study of the genus *Acanthagrion* (Odonata: Zygoptera). *Miscellaneous publications (University of Michigan. Museum of Zoology)* 153: 1-155

Lira-Torres I. & Briones-Salas M. 2012. Abundancia relativa y patrones de actividad de los mamíferos de los Chimalapas, Oaxaca, México. *Acta zoológica mexicana* 28: 566-585

Meurgey F. 2016. The genus *Brechmorhoga* Kirby, 1894, in the West Indies, with a proposed new status for *Brechmorhoga archboldi* (Donnelly) (Odonata; Libellulidae). *Zootaxa* 4079: 53-64

Novelo Gutiérrez R. 2009. Description of the larva of *Acanthagrion quadratum* Selys, with a key to the known larvae of the genus (Zygoptera: Coenagrionidae). *Odonatologica* 38: 321-328

Ortega-Salas H. 2017. Sistemática y distribución del género *Paraphlebia* Sélys in Hagen (Odonata: Thaumatoneuridae) en México y Centroamérica (Masters dissertation). Graduate thesis Universidad Nacional Autónoma de México. Retrieved from TESIUNAM Bibliography Online Database

Paulson D. 2009. Dragonflies and Damselflies of the West (Vol. 47). Princeton University Press

Peña-Olmedo J. 1989. Contribución al estudio del suborden Zygoptera (Insecta: Odonata) de la Sierra Norte del estado de Hidalgo, México. Graduate thesis Universidad Nacional Autónoma de México. Retrieved from TESIUNAM Bibliography Online Database

Peterson A.T., Navarro-Sigüenza A.G., Hernández-Baños B.E., Escalona-Segura G., Rebón-Gallardo F., Rodríguez-Ayala E., Figueroa-Esquivel E.M. & Cabrera-García L. 2003. The Chimalapas Region, Oaxaca, Mexico: a high-priority region for bird conservation in Mesoamerica. *Bird conservation International* 13: 227-253

SALAS-ARCOS L.D.C. 2010. *Argia pulla* Hagen in Selys, 1865 (Macho). Online on the internet, URL (10-iii-2020): http://unibio.unam.mx/irekani/handle/123456789/25915?proyecto=Irekani

VON ELLENRIEDER N. 2009. Databasing dragonflies: state of knowledge in the Neotropical region. *Agrion* 13: 58-72

WESTFALL M.J. 1992. Notes on *Micrathyria*, with descriptions of *M. pseudeximia* sp. n., *M. occipita* sp. n., *M. dunklei* sp. n. and *M. divergens* sp. n. (Anisoptera: Libellulidae). *Odonatologica* 21: 203-218

WILLIAMSON E.B. 1919. Results of the University of Michigan-Williamson Expedition to Colombia (1916–1917). IV. Notes on species of the genus *Heteragrion* Selys, with descriptions of new species (Odonata). *Occasional papers of the Museum of Zoology, University of Michigan* 68: 1-66

WILLIAMS-LINERA G., MANSON R.H. & VERA E.I. 2002. La fragmentación del bosque mesófilo de montaña y patrones de uso del suelo en la región oeste de Xalapa, Veracruz, México. *Madera y bosques* 8: 73-89

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Conclusión general

El estudio de la variación de los rasgos morfológicos y conductuales en libélulas y caballitos del diablo a diferentes niveles, ya sea a un nivel macroevolutivo, tomando en cuenta las relaciones filogenéticas entre las especies de Odonata para entender los patrones de cambio de dichos rasgos a lo largo de su historia evolutiva; o a nivel microevolutivo, averiguando como se dan estos patrones de cambio en las especies en un tiempo mucho más corto y a una escala más local, nos han permitido entender con mayor claridad como es la evolución de estos rasgos biológicos y como se relacionan con otros rasgos y con los componentes de adecuación como es el éxito de apareamiento en este Orden de insectos. Los trabajos presentados en esta tesis abordan diferentes aspectos de la evolución de rasgos morfológicos, como el tamaño corporal, y su relación con otros rasgos morfológicos, como la pigmentación a alar, y con rasgos conductuales, como sus sistemas de apareamiento o las proporciones sexuales de los adultos, además de las relaciones que existe de la variación de estos rasgos en el tiempo, ya sea en una escala macroevolutiva, evaluando la posibles causas de la variación de los rasgos de las especies actuales a partir de escenarios que pudieron ocurrir en tiempos remotos, o a una escala microevolutva, en un contexto más local en tiempo y espacio.

A nivel macroevolutivo podemos ver que en Odonata, la evolución de las proporciones sexuales de los adultos, depende en gran medida de sus sistemas de apareamiento y del momento en que estas proporciones son estimadas, además de las interacciones con el tamaño corporal o la diferencia de tamaño entre los sexos. Las proporciones sexuales estimadas en el momento que los organismos recién emergen como adultos, presentan números similares entre machos y hembras, por lo que se considera que se encuentra en equilibrio, en cambio, las proporciones estimadas cuando los adultos son sexualmente maduros muestran una mayor proporción de machos en la mayoría de las especies. El sistema de apareamiento, el tamaño corporal y la pigmentación alar son rasgos que se relacionan a estas proporciones sexuales entre los adultos recién emergidos, mientras que el sistema de apareamiento y la diferencia de tamaños corporales entre los sexos son los principales factores que afectan los patrones evolutivos de las proporciones sexuales entre los organismos sexualmente maduros. Estos resultados sugieren que es el sistema de apareamiento que presentan los machos, ya sea que defiendan un territorio para conseguir parejas o que no defiendan un área en particular y en cambio realicen una búsqueda activa de las posibles parejas, así como la relación entre el tamaño corporal de machos y de hembras, son los principales factores que se encuentran en juego para modificar o mantener las proporciones sexuales se los adultos. No obstante, se hace evidente que se requiere un mayor esfuerzo para conocer la biología y las historias de vida de un mayor número de especies de ambos subórdenes, Anisoptera y Zygoptera, ya que la cantidad de especie que use para obtener estos resultados es menor al dos por ciento de las especies que conocemos en la actualidad, y al aumentar el número de especies podríamos encontrar patrones de cambio de las proporciones sexuales con una mayor definición. Asimismo, integrar variables que puedan estar relacionadas con el mantenimiento o variación de las proporciones sexuales, como la supervivencia diferencial entre machos y hembras, podría ayudarnos a entender por qué en la mayoría de las especies hay una desviación de las proporciones con sesgos hacia los machos cuando los adultos maduran sexualmente.

Así mismo, a una escala macroevolutiva, podemos observar que la variación en la agilidad de vuelo entre los odonatos no es aleatoria, depende del suborden al que pertenezca cada especie, Anisoptera o Zygoptera, así como del sistema de apareamiento, territorial o no territorial, mostrando las adaptaciones del tamaño y de la forma del cuerpo y de las alas que han tenido las libélulas y los caballitos del diablo. Se refleja parte de la historia evolutiva de este orden de insectos, evidenciando que cada suborden ha resuelto las presiones selectivas que ha enfrentado de una manera distinta, ya que las libélulas son de mayor tamaño, además de que se perchan menos, es decir son especies voladoras más activas que los caballitos del diablo, de manera general. No obstante, hay que notar que el sistema de apareamiento influencia la agilidad de los odonatos, los machos de las especies que defienden un territorio como pre-requisito para poder conseguir parejas reproductivas, generalmente defienden sus territorios en vuelo o hacen cortejos elaborados, lo que permite una selección para el aumento de la agilidad en estas especies. Sin embargo, la muestra de especies que ocupamos en este trabajo también es reducida, por lo que aumentar el número de especies podría ayudarnos a entender de una manera más profunda como es que evoluciona este rasgo morfológico y la relación que pueda existir con la defensa de territorios y los cortejos elaborados para la atracción de parejas potenciales.

A nivel microevolutivo, presentó dos artículos en los que se investiga la variación de los tamaños corporales y su relación con los componentes reproductivos en dos especies territoriales de caballitos del diablo de la familia Calopterygidae. En una especie, *Hetaerina americana*, el tamaño corporal de los organismos que logran aparearse varía dependiendo de las presiones selectivas sexuales, generando que el apareamiento no sea aleatorio entre machos y hembras. Estos resultados sugieren que los machos pueden tener un grado de elección de sus parejas basándose en el tamaño corporal que se relaciona con el valor reproductivo de la posible pareja. Con otra especie cercanamente emparentada, *H. vulnerata*, podemos observar que el tamaño corporal de los machos aumenta conforme avanza la temporada reproductiva, así como la pigmentación alar, sin embargo, la relación entre el tamaño corporal y el tamaño alar es distinta al principio y al final de la temporada reproductiva. Este estudio sugiere que la disponibilidad de recursos durante la temporada reproductiva puede afectar el desarrollo de las larvas y de los adultos recién emergidos, generando modificaciones y selección sobre los rasgos de tamaño corporal y de pigmentación alar en los machos de esta especie, permitiendo la oportunidad para la selección sexual. Si bien generar conocimientos medite la implementación de este tipo de estos trabajos enfocados a especies particulares es de gran ayuda para entender los patrones generales y macroevolutivos, aún falta bastante esfuerzo para conocer la variación de los rasgos morfológicos y conductuales en muchas otras especies más. Los estudios que presento se realizaron con dos especies del genero *Hetaerina*, uno de los géneros más estudiados y entendidos entre los odonatologos, estas especies exhiben comportamientos territoriales, pero es poco conocido como es que se puede afectar la variación de tamaños y la diferencia sexual de tamaño en especie no territoriales, por lo que es necesario enfocarse en géneros y especies menos estudiadas para tener un panorama más amplio de las relaciones entre los rasos morfológicos y conductuales, y como estos se ven afectados por las presiones selectivas, como lo son los componentes de reproducción y de supervivencia.

Además, presento un artículo de divulgación en el cual se muestra un panorama general de los temas que se abordan en el laboratorio de biología evolutiva del Dr. Martin Alejandro Serrano Meneses, en el cual desarrollé mis estudios del doctorado, como lo es la evolución de rasgos biológicos en Odonata, en particular la evolución de la coloración corporal y alar. En este artículo muestro como es que se generan los distintos colores en entre los insectos y en particular, como se deposita la pigmentación alar en una especie de Odonata. Por último, presento un artículo sobre la diversidad de Odonata en una zona de alta prioridad para su conservación en el país, lo cual expone la necesidad de aumentar los estudios sobre la biología de este grupo de insectos. Este último trabajo que presento ha permitido proponer un seguimiento de la odonatofauna del sitio de estudio (Chimalapas-Uxpanapa, Oaxaca-Veracruz, México). El cual se encuentra en evaluación por parte de la *National Geographic* de Estados Unidos de América.

Estos seis trabajos reflejan la importancia del estudio de los rasgos biológicos y como es que podemos esclarecer la historia evolutiva de este orden de insectos, Odonata, desde distintos puntos de vista. Abordar el estudio de la evolución de diferentes rasgos morfológicos y conductuales, incluyendo el grado de parentesco entre distintas especies actuales, nos permite entender las relaciones entre dichos rasgos y como es que las especies se han adaptado al medio ambiente. Mientras que el estudio de la relación de los rasgos morfológicos y conductuales a niveles más locales en tiempo y espacio, es decir sobre una sola población, o pocas, de una especie particular, nos permite entender cómo son los mecanismos de variación de estos rasgos y relacionarlos con sus historias de vida.

Productos adicionales

In search of dragonflies from the Mexican Golden Jicara Rainforest: Odonata from the Chimalapas-Uxpanapa region.

Prpopuesta para obtener recursos economicos de la "*National Geographic Society*" de Estados Unidos de Norteamerica, para realizar exploraciones científicas para cacterizar la odonatofauna de la región Chimalapas-Uxpanapa, Oaxcaca-Veracruz (ChU)

Do marine reserves increase the prey of California sea lions and in the Pacific harbor seals?

Articulo de investigacion.

In search of dragonflies from the Mexican Golden Jicara Rainforest: Odonata from the Chimalapas-Uxpanapa region

National Geographic Grant

Project Leader Name / e-mail / Institution / Department

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3. Víctor Hugo Reynoso Rosales / vreynoso@ib.unam.mx / Universidad Nacional Autónoma de México / Colección Nacional de Anfibios y Reptiles.

4. Omar Hernandez Ordoñez / omar.hernandez@ib.unam.mx / Universidad Nacional Autónoma de México / Colección Nacional de Anfibios y Reptiles.

Project Role/Relevant Skills

1. Collector, fieldworker, identifier and desk job / Identification and collection experience in the taxa of interest.

2. Identifier and desk job / Identification, collection and writer experience in the taxa of interest.

3. Collector and fieldworker / Knowledge of the site and local people and fieldwork experience.

4. Collector and fieldworker / Knowledge of the site and local people and fieldwork experience.

Background and Relevance

Anthropogenic habitat destruction either by clandestine logging, agriculture, or by the increase in forest fires, has had a strong impact on dragonfly and damselfly (odonates) populations worldwide. It is estimated that 15% of all species of odonates could be threatened (Clausnitzer et al., 2009; Sánchez-Bayo & Wyckhuys, 2019). And even, the few that could be considered as of low concern, with the increase in temperature globally, could suffer alterations in their physiology, development, and behavior, which would cause a niche and habitat displacement for many of them (Hassall & Thompson, 2008). These threats are accentuated in the neotropical zone of Mexico, where in addition to the threats mentioned above, there is little information on the distribution and conservation status of the species that inhabit it (Paulson, 2004).

An example of this is the Chimalapas-Uxpanapa (ChU) region that crosses the border between the states of Veracruz and Oaxaca, since, despite being considered as a priority region for conservation and as part of the Mesoamerican Biological Corridor (Arriaga-Cabrera et al., 2009), it is insufficiently conserved. In the last 15 years, it has been victim of severe fires in the Santa María Chimalapa section, and excessive logging in the Uxpanapa area (Asbjornsen et al., 2005; Aguilar-López et al., 2020). In addition, it is a region so poorly studied that (Navarro-Siguenza et al., 2008), until 2018, no odonatologist had stepped on its jungle. In the summer of 2018, in a 5-day field trip within the CU region, we recognized the presence of 15 species of odonates, of which 40% of them are not listed by the IUCN, and 33% need an update of information, so we observe that the conservation status of almost 75% of the species collected is unknown (Carrillo-Muñoz & García-Miranda, in press). In addition to this, we found some species like *Hetaerina infecta*, *Heteragion alienum* and *H. tricellulare*, which by being the largest damselflies in their genre, have a higher risk of extinction (Suárez-Tovar et al., 2019).

In spite of the little conservationist attention that the odonates have received (Fisher, 2019), they are an important group, because they are used as pollution bioindicators in forest habitats, and in fresh water bodies (Rocha-Ortega et al., 2019; Šigutová et al., 2019; Vorster et al., 2020). We even consider that the study of this region can be successful, because, in the case of amphibians, local conservation initiatives have been able to house up to 73% of the endemic species of the region (Ochoa-Ochoa et al., 2009). We hope that, in the future, such will be the case for odonates. For all the above, we consider it necessary to obtain data on the diversity and distribution of odonates in the CU region, since not only is the discovery of new species possible, but it is the first step to determine the survival rate and population trend of each of them, or in other words, to preserve them. (Araújo et al., 2019; La Porta & Goretti, 2019).

Goals and Objectives

1. Describe the diversity and distribution of dragonflies and damselflies from the CU region, Mexico
    a. Collect nymphs and adults of dragonflies and damselflies from three localities within the CU region.
    b. Identify and preserve all captured specimens.
    c. Spread the distributional and taxonomic collected data, in specialized journals, scientific posters, and in local lectures within the CU community.

Methodology

We´ll make three fieldtrips to ensure that we collect the majority of Odonata species, some of them aren’t found through the year. We have already had talks whit the residents and authorities of the site, so we can confirm the entrance to the fieldwork places. The first trip will be in November, between 6 and 15, at the end of autumn, in winter we don´t go. The second will be between March 26 and April 4, in spring, and the last will be in June 4 to 13, on summer. Each fieldtrip will be in different locations to cover a larger area, but since it isn´t well explored, we cannot have known the exact places we´ll reach. We´ll need an all-terrain vehicle to get to the place where we’ll start the fieldwork, the rest of the trip will be on foot and in mules. We´ll form two teams to be able to sample a wider area, each team with two members. Aldo Carrillo-

Muñoz and Victor Reynoso-Rosales will be a team and Oscar Garcia-Miranda and Omar Hernandez-Ordoñez will be another, each team will be accompanied by one local guide.

Each fieldwork day, we will collect adults whit entomological nets near water bodies, they´ll be photographed to have the live color record, and put into glassine-paper bags until the end of the day to be process (see below). All bags will be properly labeled to match whit the field notebook, additional data will be noted (photo number, temperature, luminosity, sex, behavior and any other useful data). Any exuviae (remains of exoskeleton left after the change between juvenile and adult) found will be collected and put in a jar filled whit alcohol. Site photos and behavior videos will be taken. The larvae will be collected by stirring the bottom substrate and driving the swimming insects towards the aquatic net, they´ll be photographed and placed in jars until the end of the day (see below), all jars will be labeled and their respective data will be noted. The manipulations of all the organism will be due with nitrile globes and steel forceps to avoid pollute with grease or any other substance. At the end of the day, each organism will be euthanized introducing them inside a lethal chamber jar. Then, adults will be submerged in acetone until the next morning, the juveniles will be put in hot water and next will be submerged in acetone until the next morning, this to keep some colors and to harden the body.

Once in the lab, we´ll processed all organism put in a polyethylene bag whit a paperboard whit the corresponding data with Martin Serrano-Meneses help. They´ll be identified using stereoscopic microscope and specialized guides, and then, they´ll be deposited in the entomologic collection of the “Universidad de las Americas Puebla (UDLAP)”. For each fieldtrip we´ll generate a list of species founded and published them in specialized journals, additionally, we´ll generate a poster and a field guide to disseminate the principal outcomes to the local communities and anyone.

Methodology Justification

We already went to the work area in 2018 for a prospective fieldtrip. In that occasion we were able to collect some Odonata species in some places and we published our outcomes in a specialized scientific journal. That excursion served to refine the proposed methods. The Odonatologist (biologist who work whit dragonflies and damselflies) uses the mentioned methods whit success. We will capture organism with which we are familiar in a behavior and taxonomic way, if we capture some new species for the science, we are capable to compare it from the already known and propose a new name for it.

To be able to complete our goals, capture, identification and publication of the odonate-fauna and to fill the gap in the knowledge of the distribution in the proposed area it is necessary to make a bigger and better effort than we did in the prospective fieldtrip. We will go to little explored places, where no samples of these insects have been taken, and the area unexplored are very extensive. The proposed field methods will allow us obtain information about the richness of the Odonata species in the zone, besides that the information obtained of each species in the field, like the adult behavior or the specific place of the juvenile, will be invaluable for the science and the humanity. We not proposed new methods, but our approach is designed to extract all possible information from each species, either in the field or in the laboratory to be published, known and disseminated for people specialized in the subject and for people who are not. Also we plan disseminate our outcomes among the nearby communities so they have knowledge of their wealth and can generate local strategies for its us and conservation.

Expected Outcomes

3 Scientific publications on indexed and specialized journals, one by each fieldtrip.

1 Informative poster of the species collected and/or discovered.

Referencies

Aguilar-López, J.L., Pineda, E., Luría-Manzano, R., & Canseco-Márquez, L. (2020). Species Diversity, Distribution, and Conservation Status in a Mesoamerican Region. Tropical Conservation Science, 9(4), 1940082916670003.

Araújo, M.F.A., De-Marco, P., Juen, L., & Tôrres, N.M. (2019). Vulnerability of *Phyllocycla* Species (Odonata: Gomphidae) to Current and Planned Anthropic Activities by the Brazilian Government. Neotropical Entomology, 49, 24-32

Arriaga-Cabrera, L., Aguilar, V., Espinoza, J. M., Galindo, C., Herrmann, H., Santana, E., ... & Rosenzweig, L. (2009). Regiones prioritarias y planeación para la conservación de la biodiversidad. Capital natural de México, 2, 433-457.

Asbjornsen, H., Velázquez-Rosas, N., García-Soriano, R., & Gallardo-Hernández, C. (2005). Deep ground fires cause massive above-and below-ground biomass losses in tropical montane cloud forests in Oaxaca, Mexico. Journal of Tropical Ecology, 21, 427-434.

Carrillo-Muñoz, A.I. & García-Miranda, O. (in press). New records for the Chimalapas-Uxpanapa Region, Mexico (Odonata: Calopterygidae, Coenagrionidae, Gomphidae, Libellulidae, Megapodagrionidae, Polythoridae). *Notulae Odonatologicae*.

Clausnitzer, V., Kalkman, V.J., Ram, M., Collen, B., Baillie, J.E., Bedjanič, M., ... & Karube, H. (2009). Odonata enter the biodiversity crisis debate: the first global assessment of an insect group. Biological Conservation, 142, 1864-1869.

Fisher, M. (2019). The small, the hidden, the less-loved: conserving other species. Oryx, 53(2), 199-200.

Hassall, C., & Thompson, D. J. (2008). The effects of environmental warming on Odonata: a review. International Journal of Odonatology, 11, 131-153.

La Porta, G., & Goretti, E. (2019). Investigation on the declining Southern Damselfly (Coenagrion mercuriale, Odonata) in a Mediterranean population: survival rate and population size. Journal of Insect Conservation, 1-9.

Navarro-Siguenza, A. G., Márquez, L. C., & Monroy, H. O. (2008). Vertebrados terrestres de Los Chimalpas. Una prioridad de conservación. CONABIO Biodiversitas, 77, 10–15.

Ochoa-Ochoa, L., Urbina-Cardona, J.N., Vázquez, L.B., Flores-Villela, O., & Bezaury-Creel, J. (2009). The effects of governmental protected areas and social initiatives for land protection on the conservation of Mexican amphibians. PLoS One, 4(9), e6878.

Paulson, D.R. (2004). Critical species of Odonata in the Neotropics. International Journal of Odonatology, 7, 163-188.

Rocha-Ortega, M., Rodríguez, P., & Córdoba-Aguilar, A. (2019). Can dragonfly and damselfly communities be used as bioindicators of land use intensification? Ecological Indicators, 107, 105553.

Sánchez-Bayo, F., & Wyckhuys, K.A. (2019). Worldwide decline of the entomofauna: A review of its drivers. Biological Conservation, 232, 8-27.

Šigutová, H., Šipoš, J., & Dolný, A. (2019). A novel approach involving the use of Odonata as indicators of tropical forest degradation: When family matters. Ecological Indicators, 104, 229-236.

Suárez-Tovar, C.M., Rocha-Ortega, M., González-Voyer, A., González-Tokman, D., & Córdoba-Aguilar, A. (2019). The larger the damselfly, the more likely to be threatened: a sexual selection approach. Journal of Insect Conservation, 23, 535-545.

Vorster, C., Samways, M.J., Simaika, J.P., Kipping, J., Clausnitzer, V., Suhling, F., & Dijkstra, K.D. (2020). Development of a new continental-scale index for freshwater assessment based on dragonfly assemblages. Ecological Indicators, 109, 105819.

Budget Details

Parent Category	Grantee Budget Category	2020	2021	Total	Justification
Travel	Airfare	0	0	0	All team members are local and we will travel by land.
Travel	Vehicle Rental and Maintenance	1,500	4,500	6,000	The rent of an all-terrain vehicle plus the gasoline needed to get to the localities are of $1500. Three fieldtrips and one more with disclosure purposes.
Travel	Other Transportation	250	500	750	We will need rent of mules, and for each fieldtrip the cost is about $250. We will need it only for three fieldtrips.
Lodging/Food	Lodging	250	750	1,000	The cost of the a hotel night for four person are about $62.5, a total of four night per travel will be necessary and four trips will be done.
Lodging/Food	Food	1000	3000	4,000	The cost of food for the four team member and two local guides per travel are about $1000, including restaurants and self-service stores. A total of four trips will be done.
Equipment/Lab	Equipment and Supplies	6500	300	6,800	Clothing, camping equipment, collecting and preserving supplies, photography equipment will be necessary. The majority of equipment and supplies will be necessary since the first fieldtrip.
Equipment/Lab	Laboratory Cost	0	0	0	None laboratory cost will be necessary.
Equipment/Lab	Laboratory Tests	0	0	0	None laboratory cost will be necessary.
Compensations	Applicant and Team Members Comp	10,200	10,200	20,400	The stipend for a team member would be of $850 per month and it will be per 12 months, this stipend will cover live expenses in Mexico country. A total of two team members will recipe that stipend. The applicant, Aldo Carrillo-Muñoz, will finish their doctoral studies before starting the project and he has none contract for the duration of the project. Team member Oscar García-Miranda are a freelancer and undergraduate student collaborator without contract.
Compensations	Assistants and Consultants Comp	500	1000	1,500	The salary for a local guide each trip would be of $125 and two guides will be hired. The salary of a professional illustrator for the organism of one fieldtrip will be of $250

Evaluations	Measurements and Evaluation	0	915	915	Twenty posters will be printed for the local communities, which will cost $100. The publications of 200 copies of the field guide will be of $900
Others	Institutional Overhead	0	0	0	None institutional overhead will be necessary.
Others	Miscellaneous	500	1000	1,500	Some extra expenses will be cover, like medical emergency, equipment and supplies not considered since the beginning.

Ethical Certification

National Geographic Society has zero tolerance for bribery and corruption, and complies with all applicable laws prohibiting such conduct including the U.S. Foreign Corrupt Practices Act and the U.K. Bribery Act. Grantees may not: offer or give anything of value to a government official or any other person as an incentive to, or in exchange or as a reward for, obtaining an improper advantage for National Geographic; or give, offer, solicit or accept anything of value that is intended to induce the recipient to violate his/her duty of loyalty to his/her employer. All licenses, permits and other government permissions or approvals required to carry out a grant must be obtained through the lawful, legitimate process of the country where the grant activity occurs.

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Do marine reserves increase the prey of California sea lions and in the Pacific harbor seals?

Alejandro ARIAS-DEL-RAZO, Yolanda SCHRAMM, Gisela HECKEL, Andrea SÁENZ-ARROYO, Arturo HERNÁNDEZ, Leonardo VÁZQUEZ, Aldo Isaac CARRILLO-MUÑOZ. (2018).

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PLOS ONE

RESEARCH ARTICLE

Do marine reserves increase prey for California sea lions and Pacific harbor seals?

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Abstract

Community marine reserves are geographical areas closed to fishing activities, implemented and enforced by the same fishermen that fish around them. Their main objective is to recover commercial stocks of fish and invertebrates. While marine reserves have proven successful in many parts of the world, their success near important marine predator colonies, such as the California sea lion (*Zalophus californianus*) and the Pacific harbor seal (*Phoca vitulina richardii*), is yet to be analyzed. In response to the concerns expressed by local fishermen about the impact of the presence of pinnipeds on their communities' marine reserves, we conducted underwater surveys around four islands in the Pacific west of the Baja California Peninsula: two without reserves (Todos Santos and San Roque); one with a recently established reserve (San Jeronimo); and, a fourth with reserves established eight years ago (Natividad). All these islands are subject to similar rates of exploitation by fishing cooperatives with exclusive rights. We estimated fish biomass and biodiversity in the seas around the islands, applying filters for potential California sea lion and harbor seal prey using known species from the literature. Generalized linear mixed models revealed that the age of the reserve has a significant positive effect on fish biomass, while the site (inside or outside of the reserve) did not, with a similar result found for the biomass of the prey of the California sea lion. Fish biodiversity was also higher around Natividad Island, while invertebrate biodiversity was higher around San Roque. These findings indicate that marine reserves increase overall fish diversity and biomass, despite the presence of top predators, even increasing the numbers of their potential prey. Community marine reserves may help to improve the resilience of marine mammals to climate-driven phenomena and maintain a healthy marine ecosystem for the benefit of both pinnipeds and fishermen.

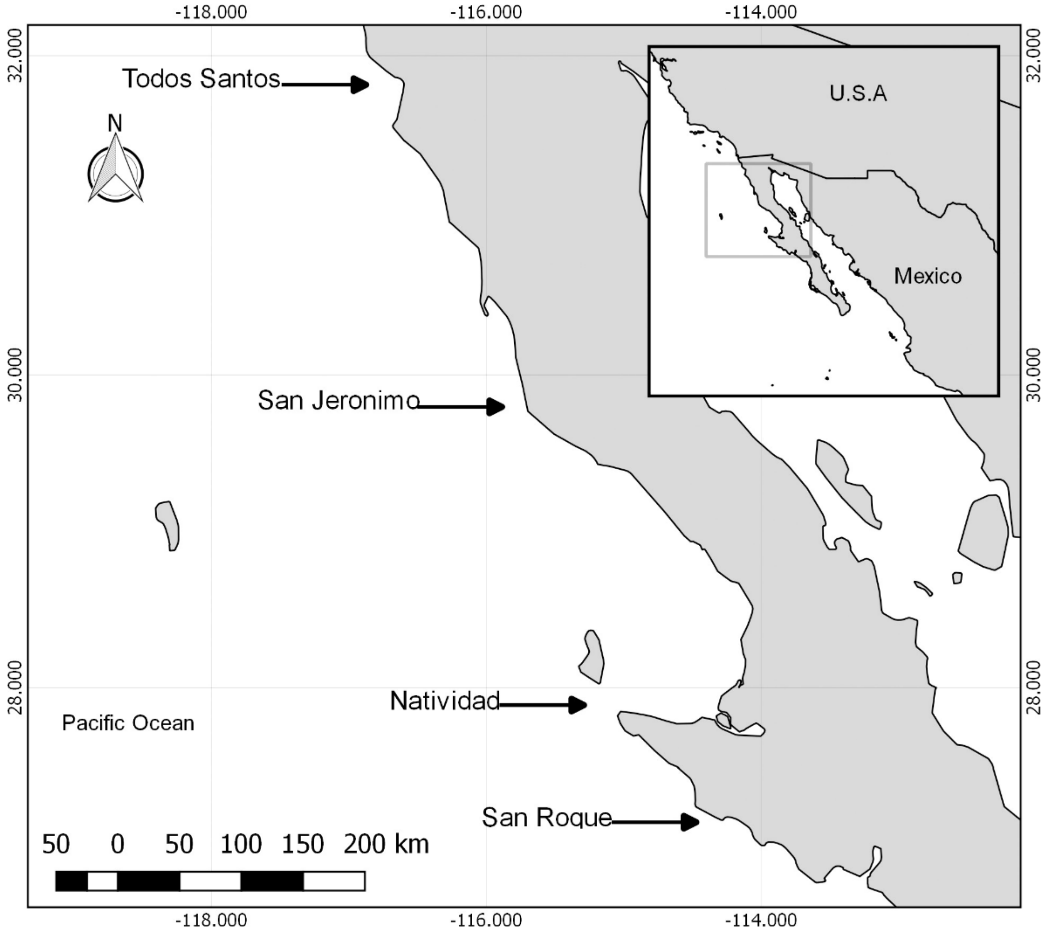

Fig 1. Study area. The study area comprised four islands in the Pacific Ocean off the west coast of the Baja California Peninsula, Mexico.

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[11]. In this region, the California sea lion's diet is composed mainly of fish and cephalopods, although it may include crustaceans, especially during abnormal oceanographic conditions [12]. Harbor seals in this region prey mainly on benthic fish, such as California lizardfish (*Synodus lucioceps*) or flatfish (*Citharichthys* spp.), but also on squid (*Doryteuthis opalescens*) and octopuses (*Octopus* spp.) [13].

Few studies have focused on the possible effects of no-take marine reserves on top predators. A comparison between undisturbed and exploited coral reef communities found significantly higher top predator (such as sharks) biomass and density on undisturbed communities [14]. Research conducted in an experimental marine reserve next to a major African penguin (*Spheniscus demersus*) colony found that the foraging effort in the colony next to the reserve reduced by 25 to 30% compared with the year prior to the establishment of the reserve. Meanwhile, no significant difference in foraging effort between both years was found in another colony which did not have an established marine reserve [15]. However, the above described

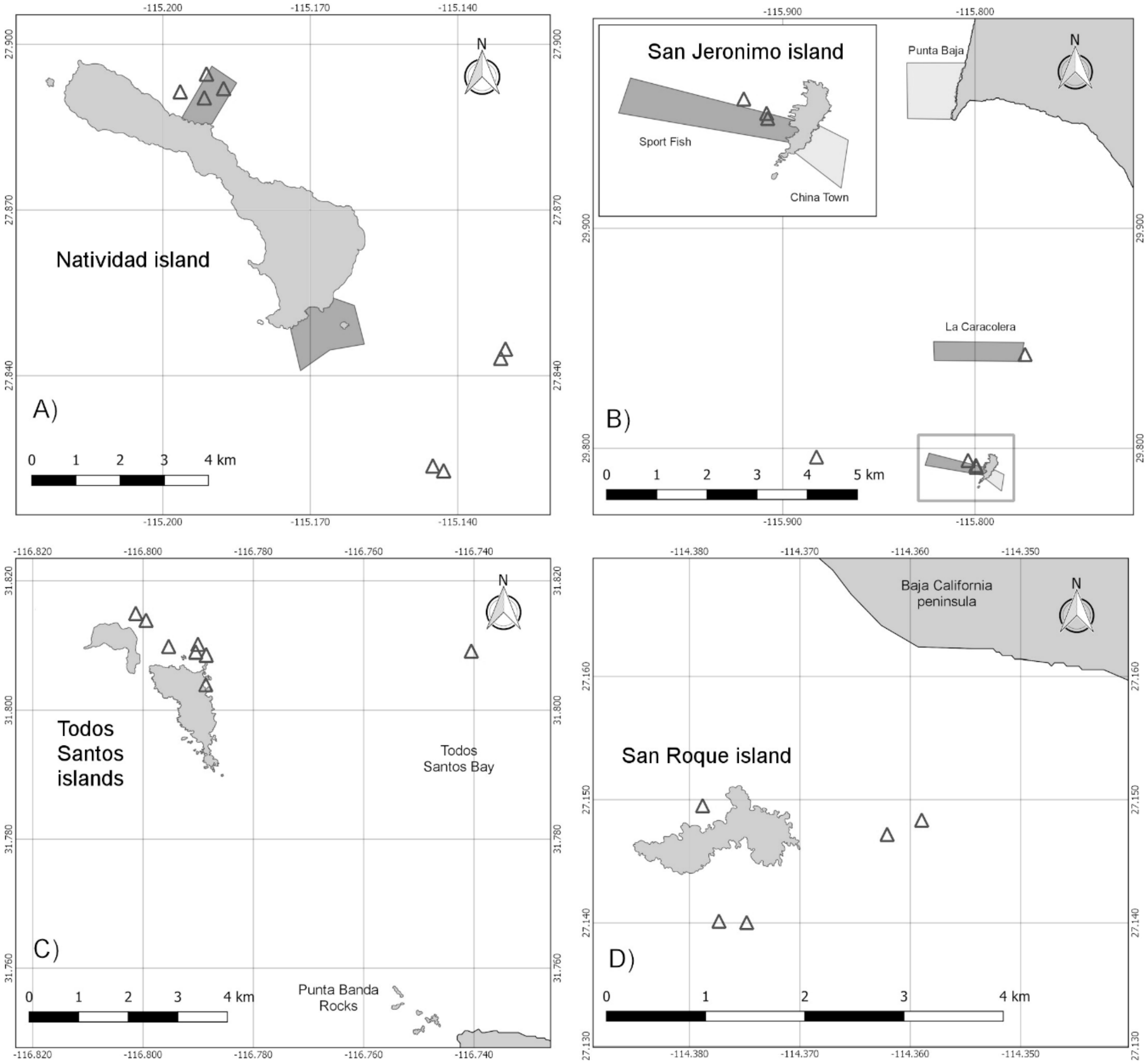

Fig 2. Survey cylinders and community marine reserves. The blue triangles represent survey cylinders, yellow polygons represent partially closed reserves and red polygons fully closed reserves. **A) Natividad island.** It has two marine reserves, with a total extraction restriction implemented in 2006. **B) San Jeronimo Island**. There are four reserves near the island, established in 2014. **C) Todos Santos islands.** Although the local fishing cooperative has exclusive rights to the waters close to the islands' shores, an aquaculture company also operates to the southeast of Todos Santos South. Industrial fishing is also carried out in the waters of the bay, with a commercial and fishing port nearby. **D) San Roque Island.** There are only harbor seals on this island. However, there is a significant sea lion colony on nearby Asuncion Island, 9.5 km to the southeast.

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findings should not lead to the expectation that all species increase in abundance and biomass within marine reserves. Given that no take-reserves change the dynamics of the community, it should be expected that some species increase in abundance while others decline [16]. Red abalone (*Haliotis rufescens*) has shown lower densities in marine reserves where sea otters

(*Enhydra lutris*) have been reintroduced [17]. In conclusion, these studies have shown that protected or undisturbed areas tend to favor top predators like harbor seals and other pinnipeds, which should be taken into account when planning marine reserves for the recovery of depressed stocks [11].

In light of the above, we hypothesized that the marine reserves established at *Natividad* and *San Jeronimo* islands may be effective in increasing overall fish biomass and diversity, despite the pinniped populations on the islands.

To assess the above described effect, we compared fish biomass and diversity around four islands off the west coast of the Baja California peninsula: two without marine reserves–Todos Santos and San Roque; San Jeronimo, with reserves established the same year as our surveys were conducted; and, Natividad, with reserves that had been established for eight years (since 2006) by the time our surveys were conducted in 2014. There are important harbor seal colonies at all of these sites, while, on Todos Santos, San Jeronimo and Natividad, California sea lion colonies are also found.

Methods

We conducted underwater surveys around four islands along the Pacific coast of the Baja California peninsula, Todos Santos, San Jeronimo, Natividad and San Roque, with important sea lion and harbor seal rookeries [18] (Fig 1). Although there are marine reserves close to San Jeronimo and Natividad, the sampling sites chosen, both within these reserves and beyond, were over rocky substrates and in favorable visibility and wave strength conditions (Fig 2).

The surveys comprised the use of survey cylinders and were undertaken by divers from the local community, certified by COBI for scientific diving, and by scientific divers from the *Universidad Autónoma de Baja California* (UABC, or Autonomous University of Baja California). Each diver stood suspended on the water column at a depth of between 9 and 22 meters, and took a series of point-count snapshots of the fish present in the 360° around their position and at a distance of up to two meters, for 15 minutes [19]. The divers recorded location coordinates, date, depth, water temperature, species, number of individuals and size. This process was repeated between 11 and 16 times per island.

For macroinvertebrates, a different team of divers swam close to the bottom, at a depth of between 11 and 24 meters, following a 30-meter long transect ($60m^2$) and recording all macroinvertebrates one meter to the right and left of the transect line [19], with between 14 to 16 repetitons per island. It should be noted that bad weather conditions forced the suspension of the survey on San Jeronimo Island after only five surveys. All surveys were conducted during August and September 2014.

Fish biomass

Fish biomass was calculated using Froese and Thorson's [20] Bayesian approach for estimating length-weight relationships in fish, with A and B parameters for the length-weight relationships obtained from FishBase [21].

To compare prey biomass, the potential prey species for pinnipeds were selected based on prior dietary studies conducted in this region on California sea lions [12] and harbor seals [13]. Invertebrate prey, such as squid and octopus, were excluded here because we did not have data on such species.

Data analysis

In order to analyze the effect of the implementation of marine reserves on the biomass around these islands, we constructed different generalized linear mixed models (GLMM) [22]. Fish biomass was used as the response variable, while years of protection (the period of time elapsed from the establishment of the reserves to the sampling date) and protection status (whether the site was inside or outside a reserve) were used as explanatory variables. The sampling sites around the islands were used as the random factor. We constructed different models with different combinations of variables and selected the best according to its Akaike Information Criterion (AIC) score, with the lowest score indicating a more parsimonious model [23]. We used R (*A Language and Environment for Statistical Computing*) version 3.5.3 to construct the GLMMs [24].

The same analysis was employed using the biomass of potential California sea lion and Pacific harbor seal prey, but using a filter database of fish biomass constructed according to the known prey of each pinniped species [12, 13, 25, 26].

Species accumulation curves were used to measure fish and macroinvertebrate species richness, while Simpson's diversity index, using EstimateS 9.1, measured biodiversity [27]. We chose the Simpson index because it tends to be more sensitive than the Shannon index to the dominant species in a community [28], thus complementing the species richness information provided by the species accumulation curve. We compared the fish diversity around each island via one-way ANOVA tests and *a posteriori* Tukey tests, while a Kruskal-Wallis test was applied to establish invertebrate diversity. Minitab 17 was used for both analyses [29].

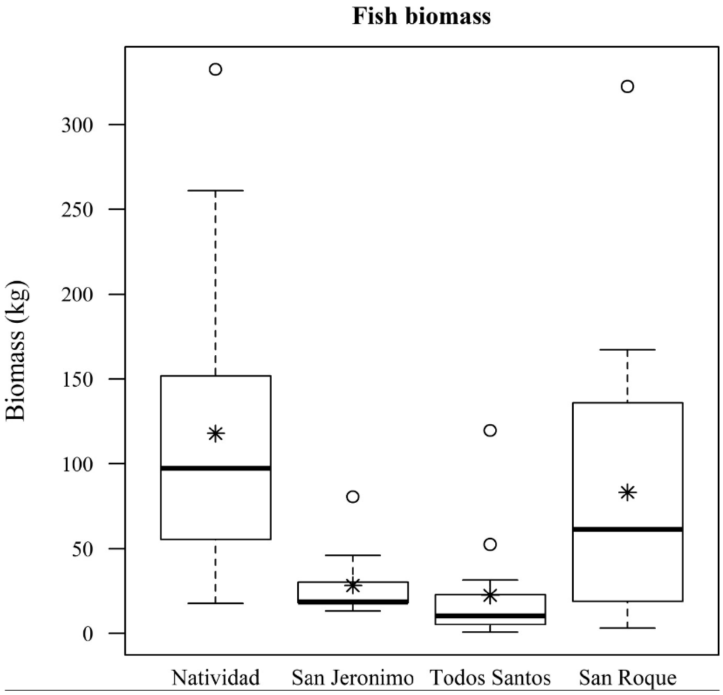

Fig 3. Fish biomass. The asterisks represent the mean fish biomass, the circles represent the outliers, the whiskers represent upper and lower quartiles, the boxes represent the interquartile range and the bands inside the boxes represent the median.

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Results

Fish biomass

Fish biomass, considering all species and survey points both within marine reserves and beyond (n = 55), was highest around Natividad Island, followed by San Roque, San Jeronimo and Todos Santos islands (Fig 3). After filtering the results for known California sea lion fish prey, biomass was also found to be higher around Natividad than around Todos Santos and San Jerónimo (Fig 4). We did not include San Roque because there are no sea lion rookeries there. Both Natividad and San Jeronimo recorded similarly high mean numbers for the fish biomass of potential Pacific harbor seal prey, although the confidence intervals are very wide, while San Roque and Todos Santos presented very low biomass levels (Fig 5).

In all models, the data show a gamma distribution. According to its AIC value, the best model uses the fish biomass as the response variable and both the level of protection of the site (inside or outside of a reserve) and the years since the marine reserve were established as explanatory variables (S1 Table). The results show that the age of the marine reserve around the islands is a significant factor with a positive trend (p = 0.037), while the protection of the site (inside or outside of the reserve) was not (p = 0.694; S2 Table). This means that the biomass around Natividad Island, with marine reserves established eight years prior to the survey, tended to be higher than that found around San Jeronimo, protected for one year, or Todos Santos and San Roque, which lack this protection entirely. Additionally, the protection status

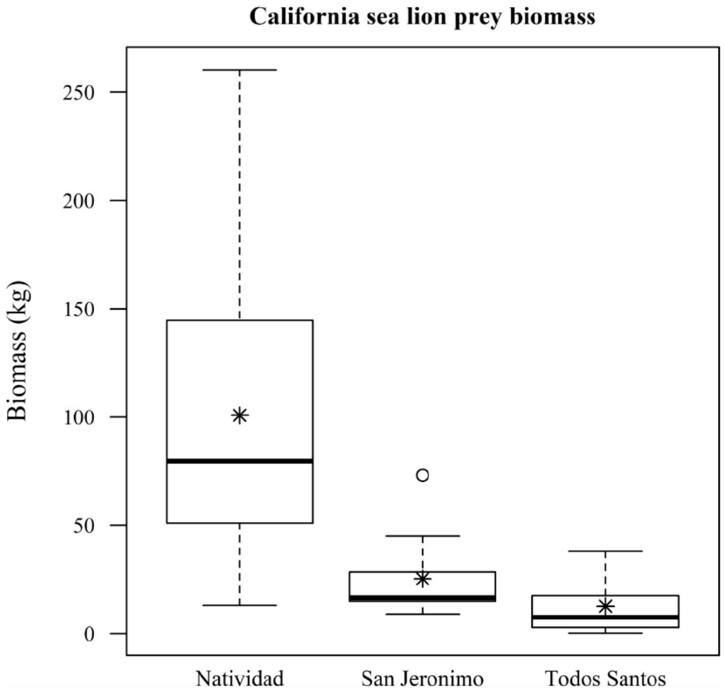

Fig 4. Fish biomass of potential California sea lion prey. Only the reported fish prey of California sea lions are included. The asterisks represent the mean fish biomass, the circles represent the outliers, the whiskers represent upper and lower quartiles, the boxes represent the interquartile range and the bands inside the boxes represent the median.

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Results

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Fish biomass, considering all species and survey points both within marine reserves and beyond (n = 55), was highest around Natividad Island, followed by San Roque, San Jeronimo and Todos Santos islands (Fig 3). After filtering the results for known California sea lion fish prey, biomass was also found to be higher around Natividad than around Todos Santos and San Jerónimo (Fig 4). We did not include San Roque because there are no sea lion rookeries there. Both Natividad and San Jeronimo recorded similarly high mean numbers for the fish biomass of potential Pacific harbor seal prey, although the confidence intervals are very wide, while San Roque and Todos Santos presented very low biomass levels (Fig 5).

In all models, the data show a gamma distribution. According to its AIC value, the best model uses the fish biomass as the response variable and both the level of protection of the site (inside or outside of a reserve) and the years since the marine reserve were established as explanatory variables (S1 Table). The results show that the age of the marine reserve around the islands is a significant factor with a positive trend (p = 0.037), while the protection of the site (inside or outside of the reserve) was not (p = 0.694; S2 Table). This means that the biomass around Natividad Island, with marine reserves established eight years prior to the survey, tended to be higher than that found around San Jeronimo, protected for one year, or Todos Santos and San Roque, which lack this protection entirely. Additionally, the protection status

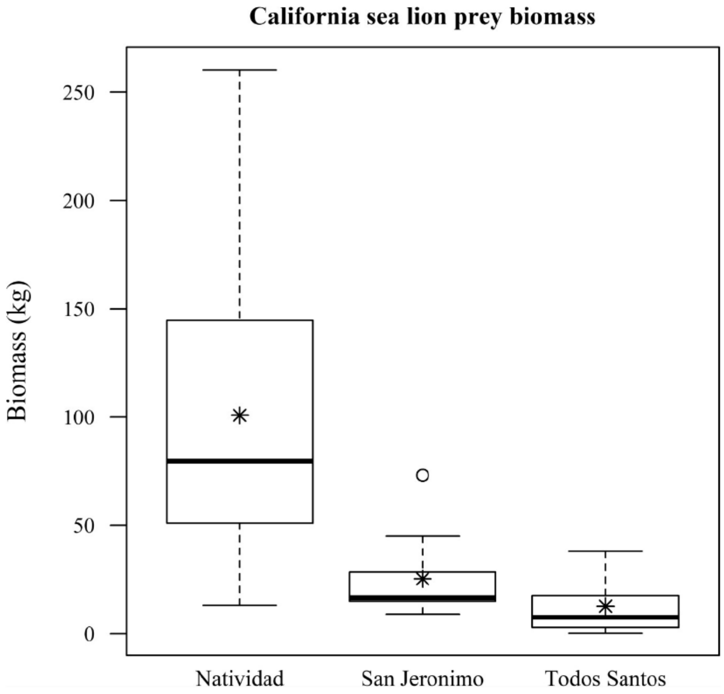

Fig 4. Fish biomass of potential California sea lion prey. Only the reported fish prey of California sea lions are included. The asterisks represent the mean fish biomass, the circles represent the outliers, the whiskers represent upper and lower quartiles, the boxes represent the interquartile range and the bands inside the boxes represent the median.

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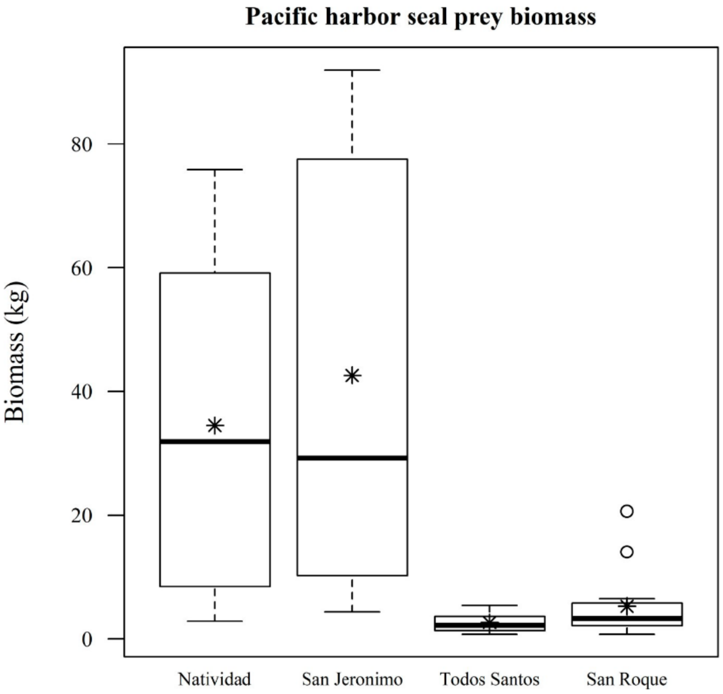

Fig 5. Fish biomass of potential Pacific harbor seal prey. Only the reported fish prey of Pacific harbor seals are included. The asterisks represent the mean fish biomass, the circles represent the outliers, the whiskers represent upper and lower quartiles, the boxes represent the interquartile range and the bands inside the boxes represent the median.

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of the sampling site had little effect on fish biomass levels, which tended to be similar both inside and outside the marine reserves. This could potentially mean that there is a spillover effect around the marine reserves. The model showed a significant intercept (p = 0.001), indicating that biomass around islands without a reserve is different from zero.

After filtering the biomass of potential California sea lion prey, the best model used potential prey biomass as the response variable, and the protection of the sampling site and the years since the establishment of the marine reserve as explanatory variables (Table 1). In this model, we eliminated the results from San Roque because there are no California sea lion rookeries on that island. The results show that the age of the marine reserves had a significant positive effect on prey biomass (p = 0.001), while the protection status of the site did not (p = 0.938). The intercept was significant (p = 0.001; Table 2). As with the general biomass, this result shows

Table 1. GLMMs of the fish biomass of California sea lion prey against years and protection of the site.

Fixed effects	AIC	Deviance	Variance of Residuals
Anything	52528	52520	11.295
Years of protection	52522	52512	11.29
Years and protection of the site	52524	52512	11.29
Protection of the site	52530	52520	11.295
Years, protection and interaction	52526	52512	11.284

https://doi.org/10.1371/journal.pone.0218651.t001

Table 2. GLMMs of the fish biomass of California sea lion prey, with years of protection and protection of the site as response variables.

Fixed effects	Estimate (s. e.)	*t*	*P*
Intercept	4.368 (0.395)	11.062	0.001
Protection of the site	-0.028 (0.368)	0.077	0.938
Years of protection	0.751 (0.187)	4.008	0.001
Variance of random effects			
Residual		11.29	
Sampled spot nested in island		9.69	
Island		0	

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that islands with older marine reserves, such as Natividad, have a significantly higher prey biomass, which, in this research, was similar both inside and outside the reserves.

The best model for the biomass of potential Pacific harbor seal prey had the same variables, with none of the explanatory variables having a significant effect on the biomass of potential prey. In this model, it was the protection of the sample site that had a small negative effect on biomass (Tables 3 and S3).

Biodiversity

Although fish species richness was higher around Natividad than the other islands (n = 31), the sampling effort here was also higher, as can be seen in the fish species accumulation curve (Fig 6). The effort around San Jeronimo was reduced due to weather conditions; however, considering the minimum common sampling effort, San Jeronimo presented the highest level of richness (n = 30). Fish diversity, which considers both richness and evenness, was significantly higher around Natividad than around the rest of the islands, according to Simpson's index (F3,51 = 90.05, $P < 0.001$), followed by San Roque, San Jeronimo and Todos Santos, respectively.

Finally, the macroinvertebrate species accumulation curve (Fig 7) shows that Natividad presented the highest species richness value. Although the highest number of sampling points were applied on Natividad, richness plateaus were obtained around San Roque and Todos Santos islands before records were taken at the last sample point, meaning that sufficient sampling effort was undertaken. We were unable to estimate species richness around San Jerónimo Island due to adverse weather conditions. In terms of the Simpson diversity index, San Roque presented the highest mean (4.035, 95% CI = 3.817–4.13), followed by Todos Santos (3.515, 95% CI = 3.222–3.58) and Natividad (3.2, 95% CI = 3.079–3.217), with a significant difference found between at least in one of the means (Kruskal-Wallis, H = 23.98, DF = 2, $P < 0.001$). San Jerónimo was not included in the surveys for the Simpson diversity test due to the low number of survey sites.

Table 3. GLMMs of the fish biomass of Pacific harbor seal prey, with years of protection and protection of the site as response variables.

Fixed effects	Estimate (s. e.)	*t*	*P*
Intercept	3.511 (1.069)	3.284	**0.001**
Protection of the site	-0.624 (0.332)	1.881	0.059
Years of protection	0.665 (0.561)	1.185	0.236
Variance of random effects			
Residual		3.524	
Sampled spot nested in island		2.273	
Island		3.685	

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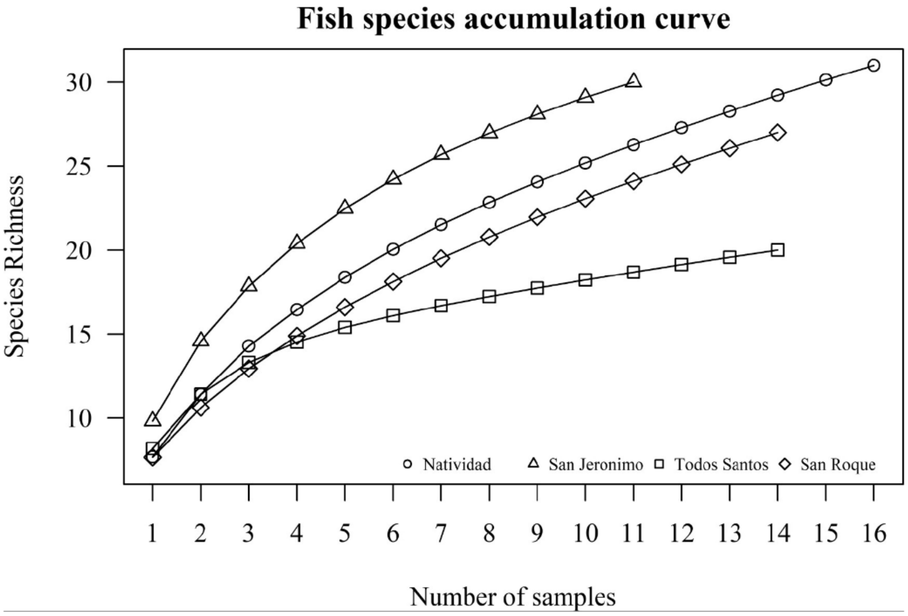

Fig 6. Fish species accumulation curve. The number of survey cylinders are represented on the X axis and the accumulation of new species recorded after taking survey data at each sample point is represented on the Y axis as species richness. Each line represents a different island.

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Discussion

The results of the present research showed that, even though similar exploitation methods and rates are found on all four islands studied here [7], fish biomass and diversity around Natividad Island is clearly superior to the other islands. According to our model, this may be attributed to the fact that marine reserves have been established around this island since 2006, given that the age of the reserve was a significant factor in the higher fish biomass found there. Moreover, the protection provided by the reserve was not significant, which may indicate a spillover effect, where biomass and diversity are similar both inside and outside these reserves. In other parts of the world, fish reserves have been demonstrated to increase the abundance and size of the fish inside them [2, 30, 31]. However, in order for them to be a successful management and conservation tool, it is necessary that there is a flow of individuals from the reserves to the surrounding fishing areas, which has proven difficult to verify [32]. In contrast, many studies have found increased abundance, over time, both inside marine reserves and in adjacent fishing areas over time [32–34]. The spillover success of different species depends on their characteristics, wherein they may be either density-dependent or independent because of random, migratory or behavioral movements [32].

In the case of San Jeronimo, the date on which the reserve was established was too close to the survey dates to show any significant improvements over islands without reserves. However, as with the results for Natividad, an effective increase both in biomass and in biodiversity may also be expected at San Jeronimo Island.

All four islands under study have important harbor seal colonies, with colony sizes of 473 individuals on Todos Santos, 523 on San Jeronimo, 724 on Natividad and 633 on San Roque

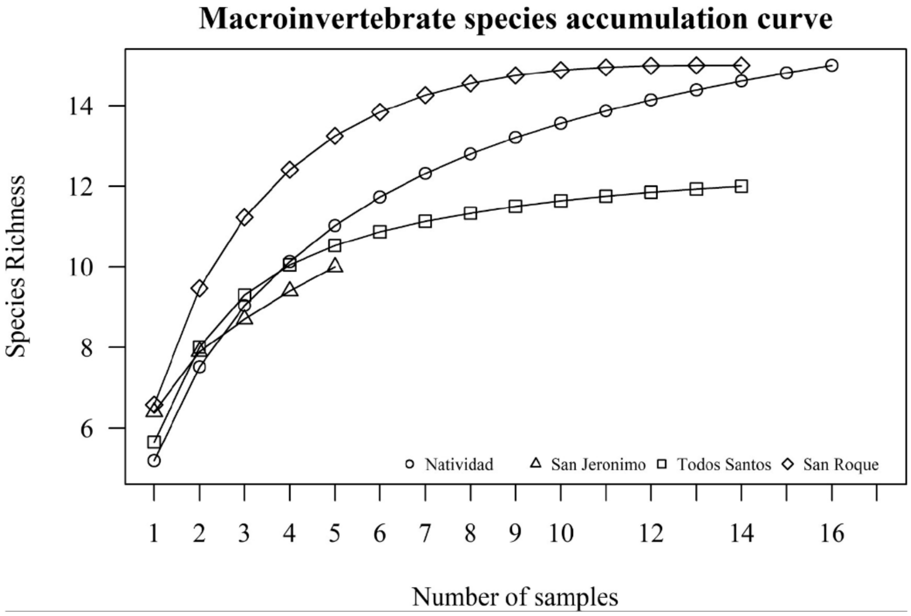

Fig 7. Macroinvertebrate species accumulation curve. The number of macroinvertebrate transects are represented on the X axis and the accumulation of new species recorded after taking survey data at each sample point is represented on the Y axis as species richness. Each line represents a different island. Only five transects were applied around San Jeronimo Island due to bad weather conditions.

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recorded during the pupping season of 2009 [35]. Their diet on these islands consists mainly of benthic fish, such as California lizardfish (*Synodus lucioceps*), flatfish (*Citharichthys* spp.) rockfish (*Sebastes* spp.) and toadfish (*Porichthys notatus*), as well as octopus (*Octopus* spp.) and squid (*Doryteuthis opalescens*) [13]. When the marine reserves were established, local fishermen were concerned that pinniped foraging would prevent the recovery of target species in the reserves. However, we found that the biomass of potential harbor seal prey was higher around both Natividad and San Jeronimo compared to San Roque and Todos Santos, despite Natividad having the largest harbor seal rookery [36].

California sea lion colonies are typically larger than harbor seal colonies because their population in the region is much higher, estimated at 33,447 in 2010 [37], compared to 4,862 harbor seals in 2009 [38]. In 2010, the size of sea lion colonies on these islands was 449 on Todos Santos, 1,371 on San Jeronimo, and 1,176 on Natividad. While there are no sea lions on San Roque, 2,186 sea lions were recorded that year on Asuncion Island, 9.5 km to the southeast [39]. With little preference for specific prey, the California sea lion has sometimes been called an opportunistic predator [40]. However, a review of diet studies and databases in this region revealed the California sea lion to be a plastic specialist, meaning that it consumes many different resources in low quantities and few resources with a high frequency [12]. California sea lions consume more than 137 species of fish and 24 mollusk species, mainly cephalopods [12].

Our results showed that the biomass of potential California sea lion prey was higher around Natividad, with the model showing a significant effect of the age of the reserves, with Natividad the only island with eight-year-old reserves at the time of sampling. Around San Jeronimo, which has a colony with a similar size, the prey biomass mean was up to four times lower. This indicates that the implementation of marine reserves has not only the potential to recover fisheries production, but also to increase potential pinniped prey biomass and overall fish diversity, thus potentially benefiting pinniped populations [1, 9].

The last point discussed above is important in two main ways. Firstly, it would give these predators more resilience to cope with adverse oceanographic conditions, such as the El Niño-Southern Oscillation [38, 39], minimum oxygen zones, or other climate change effects, such as the movement of certain prey away from high-temperature water masses. The marine reserves around Natividad have already been proven to increase ecosystem resilience to minimum oxygen zones caused by climate effects [4]. Secondly, the above discussed effect has the potential to reduce conflicts between fishermen and pinnipeds, with severe conflicts occurring between them around the world, threatening both these marine mammals' existence and the fisheries' income [40]. There seems to be a trend, in that the more exploited a marine ecosystem is, the bigger the conflicts become [7, 41–44]. The fishermen of the cooperatives under study report conflicts with the California sea lion and, to a lesser extent, with the harbor seal populations. These animals tend to damage fishing gear, including lobster traps, and scare fish from the nets, although these conflicts are on a much lower scale than in other parts of the world. Comparing the four islands studied in the present research, Natividad had a lower percentage of complaints from fishermen about such conflicts than the other three [7].

In conclusion, our results show that overall fish biomass and diversity increases after marine reserves have been established for a number of years, even in the presence of large pinniped colonies. These reserves also have the potential to mitigate climate-induced stress on their populations, as well as increasing potential prey biomass; therefore, they may help pinniped population health and, by increasing prey availability, may reduce competition for common resources between fishermen and pinnipeds.

Supporting information

S1 Table. GLMMs of all fish biomass against years and protection of the site.
(PDF)

S2 Table. GLLMs using years of protection and protection of the site as response variables.
(PDF)

S3 Table. GLMMs of the fish biomass of all harbor seal prey against years and protection of the site.
(PDF)

Acknowledgments

Fieldwork was conducted under permits issued by SEMARNAT (SGPA/DGVS/04007/08, SGPA/DGVS/04188/09, SGPA/DGVS/09252/09, SGPA/DGVS/03550/10, SGPA/DGVS/03551/10). We thank the divers and cooperative board members from *Cooperativa de Producción Pesquera California de San Ignacio*, *Cooperativa Buzos y Pescadores de la Baja California*, and *Cooperativa Ensenada*, as well as the *Unidad de Producción Acuícola de Peces Pacífico Aquaculture S de RL de CV* for their cooperation and help. Special thanks to P. Durazo, J.C. Villaseñor and P. Orta for their help during fieldwork. We thank Benjamin Stewart for proofreading this work.

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Validation: Yolanda Schramm.

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Writing – original draft: Alejandro Arias-Del-Razo.

Writing – review & editing: Yolanda Schramm, Gisela Heckel, Andrea Sáenz-Arroyo, Arturo Hernández, Leonardo Vázquez, Aldo I. Carrillo-Muñoz.

References

1. Hooker SK, Gerber LR. Marine reserves as a tool for ecosystem-based management: The potential importance of megafauna. BioScience. 2004; 54(1): 27–39.
2. Halpern BS. The impact of marine reserves: do reserves work and does reserve size matter? Ecol Appl. 2003;S117–S37.
3. Roberts CM, Bohnsack JA, Gell F, Hawkins JP, Goodridge R. Effects of marine reserves on adjacent fisheries. Science. 2001; 294(5548): 1920–3. https://doi.org/10.1126/science.294.5548.1920 PMID: 11729316
4. Micheli F, Saenz-Arroyo A, Greenley A, Vazquez L, Espinoza Montes JA, Rossetto M, et al. Evidence that marine reserves enhance resilience to climatic impacts. PLoS ONE. 2012; 7(7): e40832. https://doi.org/10.1371/journal.pone.0040832 PMID: 22855690
5. Arias-Del Razo A, Schramm Y, Heckel G, Milanés-Salinas Á, García-Capitanachi B, Lubinsky-Jinich D, et al. Distribution of four pinnipeds (*Zalophus californianus, Arctocephalus philippii townsendi, Phoca vitulina richardii, and Mirounga angustirostris*) on islands off the west coast of the Baja California Peninsula, Mexico. Aquat Mamm. 2017; 43(1): 40.
6. McCay BJ, Micheli F, Ponce-Díaz G, Murray G, Shester G, Ramirez-Sanchez S, et al. Cooperatives, concessions, and co-management on the Pacific coast of Mexico. Mar Policy. 2014; 44: 49–59.
7. Arias-Del-Razo A. Factores que determinan la preferencia de hábitat de los pinnípedos en las islas del Pacífico de Baja California. Doctoral dissertation, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California. 2016. Available from: https://biblioteca.cicese.mx/catalogo/tesis/ficha.php?id=24518
8. Morales-Bojórquez E, Muciño-Díaz MO, Vélez-Barajas JA. Analysis of the decline of the abalone fishery (*Haliotis fulgens and H. corrugata*) along the west central coast of the Baja California Peninsula, Mexico. J Shellfish Res. 2008; 27(4): 865–70.
9. Villaseñor-Derbez JC, Faro C, Wright M, Martínez J, Fitzgerald S, Fulton S, et al. A user-friendly tool to evaluate the effectiveness of no-take marine reserves. PLoS ONE. 2018; 13(1): e0191821. https://doi.org/10.1371/journal.pone.0191821 PMID: 29381762
10. DOF (Mexican Federal Register). ACUERDO por el que se establece una red de dos zonas de refugio pesquero parciales permanentes en aguas marinas de jurisdicción federal adyacentes a Isla Natividad, ubicada en el Municipio de Mulegé, en el Estado de Baja California Sur. Diario Oficial de la Federación-SAGARPA. 7 June 2018. Available from: http://dof.gob.mx/nota_detalle.php?codigo=5525396&fecha=07/06/2018 Cited 31 July 2018.

11. Lance MM, Chang WY, Jeffries SJ, Pearson SF, Acevedo-Gutiérrez A. Harbor seal diet in northern Puget Sound: implications for the recovery of depressed fish stocks. Marine Ecology Progress Series. 2012; 464: 257–271.

12. Espinosa de los Reyes-Ayala MG. Variabilidad espacial de la dieta del lobo marino de California (*Zalophus californianus californianus, Lesson 1828*). M.Sc. Thesis, Centro de Investigación Científica y de Educación Superior de Ensenada. 2007. Available from: https://biblioteca.cicese.mx/catalogo/tesis/ficha.php?id=17748

13. Durazo-Rodríguez RP. Variación espacio-temporal de los hábitos alimentarios de la foca de puerto (*Phoca vitulina richardii*) en México. M.Sc. Thesis, Universidad Autónoma de Baja California. 2015. Available from: https://drive.google.com/file/d/0ByZyFBRL67c0TzdoeDl3QzV1dGs/view

14. Sandin SA, Smith JE, DeMartini EE, Dinsdale EA, Donner SD, Friedlander AM, et al. Baselines and Degradation of Coral Reefs in the Northern Line Islands. PLOS ONE. 2008; 3(2):e1548. https://doi.org/10.1371/journal.pone.0001548 PMID: 18301734

15. Pichegru L, Grémillet D, Crawford RJM, Ryan PG. Marine no-take zone rapidly benefits endangered penguin. Biology letters. 2010; 6(4):498–501. https://doi.org/10.1098/rsbl.2009.0913 PMID: 20147313

16. Soulé ME, Norse EA, Crowder LB. Marine Conservation Biology: The Science of Maintaining the Sea's Biodiversity: Island Press; 2005.

17. Fanshawe S, Vanblaricom GR, Shelly AA. Restored Top Carnivores as Detriments to the Performance of Marine Protected Areas Intended for Fishery Sustainability: a Case Study with Red Abalones and Sea Otters. Conserv Biol. John Wiley & Sons, Ltd (10.1111); 2003; 17: 273–283. https://doi.org/10.1046/j.1523-1739.2003.00432.x

18. Fernández Martín EM. Fenología de los nacimientos y de la muda de *Phoca vitulina richardsi* (Gray, 1864) en el Estero de Punta Banda, B.C., México. M.Sc. Thesis, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California. 2012. Available from: https://biblioteca.cicese.mx/catalogo/tesis/ficha.php?id=18992

19. Shuman CS, Saenz Arroyo A, Dawson C, Luna MC. Manual de introduccióon ReefCheck California: Guía para el monitoreo del bosque de Sargazo en la Península de Baja California. Pacific Palisades, California, USA: ReefCheck Foundation; 2010.

20. Froese R, Thorson JT, Reyes RB. A Bayesian approach for estimating length-weight relationships in fishes. J Appl Ichthyol. 2014; 30(1): 78–85.

21. Froese R, Pauly D. FishBase; 2017 [cited 14 April 2017]. Available from: http://www.fishbase.org.

22. McCulloch CE, Neuhaus JM (2005) Generalized Linear Mixed Models. Encyclopedia of Biostatistics.

23. Akaike H (1973) Information theory as an extension of the maximum likelihood principle–In: Second International Symposium on Information Theory (Eds) BN Petrov, F. Csaki. BNPBF Csaki Budapest: Academiai Kiado.

24. R Core Team (2019) R: A Language and Environment for Statistical Computing. 3.5.3 ed. Vienna, Austria: R Foundation for Statistical Computing.

25. Alamán de Regules R (2014) Hábitos alimentarios de la foca de Puerto, Phoca vitulina richardsi, en la Bahía Todos Santos, Baja California, México [Masters]. Ensenada, Baja California, México: Universidad Autónoma de Baja California. 79 p.

26. Elorriaga-Verplancken F, Morales-Luna L, Moreno-Sánchez XG, Mendonza-Salas I (2013) Inferences on the Diet of the Eastern Pacific Harbor Seal (Phoca vitulina richardii) at the Southern End of Its Distribution: Stable Isotopes and Scats Analyses. Aquatic Mammals 39: 415–421.

27. Colwell R. EstimateS: Biodiversity Estimation Software. 2013 [cited 8 February 2018]. Available from: http://viceroy.eeb.uconn.edu/estimates/

28. Nagendra H (2002) Opposite trends in response for the Shannon and Simpson indices of landscape diversity. Applied geography 22: 175–186.

29. Minitab Inc: Minitab. Version 17. 2017 [cited 10 March 2018]. Available from: http://www.minitab.com

30. Côté IM, Mosqueira I, Reynolds JD. Effects of marine reserve characteristics on the protection of fish populations: a meta-analysis. J Fish Biol. 2001; 59: 178–89.

31. Gell FR, Roberts CM. The fishery effects of marine reserves and fishery closures. Washington, DC: WWF-US; 2002.

32. Abesamis RA, Russ GR. Density-dependent spillover from a marine reserve: long-term evidence. Ecol Appl. 2005; 15(5): 1798–812.

33. Kaunda-Arara B, Rose GA. Out-migration of tagged fishes from marine reef national parks to fisheries in coastal Kenya. Environ Biol Fishes. 2004; 70(4): 363–72.

34. Goñi R, Hilborn R, Díaz D, Mallol S, Adlerstein S. Net contribution of spillover from a marine reserve to fishery catches. Mar Ecol Prog Ser. 2010; 400: 233–43.

35. Lubinsky-Jinich D, Schramm Y, Heckel G. The Pacific harbor seal's (*Phoca vitulina richardii*) breeding colonies in Mexico: abundance and distribution. Aquat Mamm. 2017; 43(1): 73–81.

36. Milanés-Salinas MA. Abundancia y distribución de sitios de crianza del lobo marino de California, *Zalophus californianus*, en el Pacífico mexicano. M.Sc. Thesis, Universidad Autónoma de Baja California; 2012. Available from: https://drive.google.com/file/d/0ByZyFBRL67c0d2ZKRnBUOWRBeEk/view

37. Camacho-Ríos FJ. Estructura alimentaria y posición trófica de dos especies de otáridos, *Zalophus californianus y Arctocephalus townsendi*, en las Islas San Benito, BC, México. M.Sc. Thesis, Instituto Politécnico Nacional—Centro Interdisciplinario de Ciencias Marinas; 2004. Available from: http://www.biblioteca.cicimar.ipn.mx/oacis/Medios/tesis/camachor1.pdf

38. Trillmich F, Limberger D. Drastic effects of El Niño on Galapagos pinnipeds. Oecologia. 1985; 67(1): 19–22. https://doi.org/10.1007/BF00378445 PMID: 28309839

39. DeLong RL, Antonelis GA, Oliver CW, Stewart BS, Lowry MC, Yochem PK. Effects of the 1982–83 El Niño on several population parameters and diet of California sea lions on the California Channel Islands. In: Trillmich F, Ono K, editors. Pinnipeds and El Niño. Ecological Studies. 88: Springer Berlin Heidelberg; 1991. pp. 166–172.

40. Read AJ, Drinker P, Northridge S. Bycatch of marine mammals in U.S. and global fisheries. Conserv Biol. 2006; 20(1): 163–9. PMID: 16909669

41. Barlow J, Baird RW, Heyning JE, Wynne K, Manville AM, II L, et al. A review of cetacean and pinniped mortality in coastal fisheries along the west coast of the USA and Canada and the east coast of the Russian Federation. Rep Int Whaling Commn, Special Issue. 1994(15): 405–25.

42. Jamieson G, Olesiuk P. Salmon farm-pinniped interactions in British Columbia: An analysis of predator control, its justification and alternative approaches (Canadian Science Advisory Secretariat Research Document 2001/142). Fisheries and Oceans Science: Ottawa, Canada; 2001.

43. Wright BE, Riemer SD, Brown RF, Ougzin AM, Bucklin KA. Assessment of harbor seal predation on adult salmonids in a Pacific Northwest estuary. Ecol Appl. 2007; 17(2): 338–51. PMID: 17489243

44. Gruber CP. Social, economic, and spatial perceptions of gray seal (Halichoerus grypus) interactions with commercial fisheries in Cape Cod, MA. M.Sc. Thesis, Duke University. 2014. Available from: https://dukespace.lib.duke.edu/dspace/handle/10161/8473