UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

Instituto de Ecología

COEVOLUCIÓN ANTAGONISTA E IMPRONTA GENÉTICA EN EL PEZ MATROTRÓFICO *Girardinichthys multiradiatus*

T E S I S

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

P R E S E N T A:

YOLITZI SALDÍVAR LEMUS

TUTOR PRINCIPAL:

DR. CONSTANTINO DE JESÚS MACÍAS GARCIA

INSTITUTO DE ECOLOGÍA

MIEMBROS DEL COMITÉ TUTOR:

DR. DANIEL PIÑERO DALMAU

INSTITUTO DE ECOLOGÍA

DR. ERNESTO MALDONADO OLVIERA

INSTITUTO DE CIENCIAS DEL MAR Y LIMNOLOGÍA

CIUDAD DE MÉXICO, SEPTIEMBRE DE 2017

Coevolución antagonista e impronta genética en el pez matrotrófico

Girardinichthys multiradiatus

Antagonistic coevolution and genomic imprinting in the matrotrophic fish

Biól. Yolitzi Saldívar Lemus

Instituto de Ecología, UNAM,

Correo: yolitzi@gmail.com

Tel. 56228222, ext. 81290

Director de tesis: Dr. Constantino Macías Garcia

Comité tutor: Dr. Daniel Piñero Dalmau y Dr. Ernesto Maldonado

Jurado de examen:

Dr. José Jaime Zúñiga Vega (Facultad de Ciencias)

Dr. Carlos Cordero Macedo (Instituto de Ecología)

Dra. Hilda María Lomelí Buyoli (Instituto de Biotecnología)

Dra. Norma Angélica Moreno Mendoza (Instituto de Investigaciones Biomédicas)

Dr. Constantino Macías Garcia (Instituto de Ecología)

“If there is conflict of interest between parents and children, who share 50 per cent of each other’s genes, how much more severe must be the conflict between mates, who are not related to each other?... Since father and mother are both interested in the welfare of different halves of the same children, there may be some advantage for both of them in cooperating with each other in rearing those children. If one parent can get away with investing less than his or her fair share of costly resources in each child he will have more to spend on other children by other sexual partners, and can therefore be thought of as trying to exploit the other, trying to force the other one to invest more.“

Dawkins, The selfish gene

Índice

AGRADECIMIENTOS

AGRADECIMIENTOS ACADÉMICOS

Agradezco a mi asesor el Dr. Constantino Macías Garcia por toda su paciencia, apoyo, tiempo y esfuerzo que dedicó a mi formación académica. Sin su guía la realización de esta tesis no hubiera sido posible.

Al Dr. Jean-Phillipe Vielle Calzada, por su invaluable guía y contribución al proyecto, a mi formación académica, y al artículo producto de mi doctorado, y por permitirme formar parte de su laboratorio por tanto tiempo.

Al Dr. Michael G. Ritchie, por su valiosa aportación a mi formación académica, al proyecto y al artículo que se derivó de él, y por abrirme las puertas de su laboratorio en diversas ocasiones.

A los doctores Daniel Piñero Dalmau y Ernesto Maldonado, integrantes de mi comité tutor, por todos los comentarios y sugerencias que me dieron a lo largo de mi doctorado para mejorar el proyecto.

Al Programa de Doctorado en Ciencias Biomédicas, por los diversos apoyos económicos que recibí para la realización de estancias académicas y congresos (PAEP).

Al Consejo Nacional de Ciencia y Tecnología por la beca de doctorado (351769) y por los apoyos económicos que me otorgaron para estancias de investigación.

A PAPIIT, por el financiamiento económico que nos dieron para la realización del proyecto (IN216111).

Al Biól. Edgar Ávila Luna por su ayuda en el cuidado de los peces.

A la técnico Tanya Schneddon y al Dr. Jeff Graves por su apoyo y guía en la realización de experimentos.

A Santos García Aguilar y a Liliana Solórzano por su apoyo técnico para poder llevar a cabo los experimentos.

A la Biól. Marcela Méndez Janovitz, por proporcionarme la información de dimorfismo sexual y cortejo, y por su asesoría para la construcción de filogenias.

A Ruth E. Rodríguez Tejeda por sus valiosos comentarios a esta tesis.

A Rebecca Boulton por sus comentarios y revisión del inglés del capítulo 3 de esta tesis.

A los integrantes de mi jurado de examen por los valiosos comentarios que me hicieron para mejorar mi tesis.

AGRADECIMIENTOS PERSONALES

Agradezco enormemente a mi familia. A mis papás por su cariño, su apoyo incondicional, sus porras y por siempre impulsarme a superarme. A mi hermana Itzel, por su increíble compañía y por el apoyo que me ha brindado toda la vida. A mis primos Itza y Chris por los buenos momentos, por los ánimos que siempre me han dado, y por escuchar mis frustraciones.

A Thelma, Azya, Jess, Rodrigo, Ruth, Jean, Aarón, por su amistad incondicional en todo momento, y porque nunca dejaron de creer en mí.

A mis amigos de la universidad por tantos buenos momentos y frustraciones del posgrado que compartimos: Elba, Radillo, Zai, Paco, Cajas, Rober, Yaxkin, Lalo, Roli, Fredo, Carlitos, Karlita, Celes, Ale, Dianis, Juan y Lara.

A mis amigos del laboratorio de Conducta Animal: Marce, Monse, Nadis, Alex, Vic, Dante, Pablo, Lau, Vero, Marina, Oscar, Lynna, Santi, Aldo, por su amistad y buenos momentos durante todos estos años.

A mis amigos del laboratorio de apomixis Vira, Isaac, Carlitos Chávez, César, Nidia, Carlitos Barragán, Gloria, Rigel, Quetz, Gloria, Sara, Juanita, Judith, Isaac Alejandro por los increíbles momentos, y por los invaluables consejos y sugerencias que siempre me dieron para mi proyecto y experimentos.

A mis amigos de St Andrews por su invaluable retroalimentación al proyecto y por los momentos increíbles que pasamos durante mis estancias doctorales: Becca, Justina, Sonia, Gil, Christine, Lilian, Mathilde, Julie, Paris, Liam, Emily, Elina, Becky, Darren, Nicky, Yi-Hsiu, Annie Hsu, Erven.

A mis amigos del tenis y la escalada, por siempre darme ánimos y por los buenos momentos: Marina, Migueli, Abeli, Migue, Dany Medina, Robert, Leo, Marchelo, Vero, Lety, Alex Guerra, Sele Guerra, Luis, Dany Montiel.

PRESENTACIÓN

La producción de descendencia viable suele ser muy costosa para los progenitores. Por ello, en organismos vivíparos donde la inversión parental se puede manipular (usualmente la materna), se espera que surjan una serie de adaptaciones (en los machos) y de contra-adaptaciones (en las hembras) que permitan incrementar y restringir respectivamente la asignación de recursos para la camada en común. Bajo este escenario de carrera armamentista, se espera que las contra-adaptaciones maternas permitan constantemente mitigar los costos en su adecuación que les fueron impuestos por las adataciones paternas.

La evolución de la viviparidad y de todas las adaptaciones madre-crío que favorecieron una transferencia de nutrientes más eficiente y gradual durante el desarrollo embrionario crearon las condiciones perfectas para que los machos, críos, o ambos, pudieran manipular la asignación de recursos maternos. Este conflicto aparentemente llevó a una coevolución antagonista entre los genomas paterno y materno en los críos, y al parecer causó que algunos genes se expresaran dependiendo de su origen parental, un fenómeno que hasta la fecha se considera exclusivo de mamíferos; sin embargo, no ha sido suficientemente explorado en otros vertebrados vivíparos matrotróficos.

Esta tesis fue desarrollada con el objetivo de tener un mejor entendimiento de la impronta genética y de su posible evolución en otros grupos de vertebrados vivíparos matrotróficos, como son los Goodeidos. Mi investigación consta de una parte teórica y una experimental. La sección teórica está conformada por dos revisiones que brindan un acercamiento al conflicto relacionado a la viviparidad y a las diversas formas de matrotrofía (capítulo 2), y a la evolución y mecanismos por los cuales se lleva a cabo la impronta genética (capítulo 3). Adicionalmente, esta tesis consta de una serie de experimentos fenotípicos (capítulo 4) y moleculares (capítulos 5 y 6), con los cuales buscamos determinar si los machos del pez vivíparo matrotrófico *Girarnichthys multiradiatus* son capaces de influir en la asignación materna de recursos durante el desarrollo embrionario, y si este conflicto sexual ha promovido la evolución de la impronta genética en el gen que codifica para el *factor de crecimiento tipo insulina 2* (*igf2*).

Cada sección de esta tesis aborda una pregunta diferente sobre la evolución del conflicto relacionado a la asignación materna de recursos durante el desarrollo embrionario y la expresión de *igf2* en goodeidos. Decidí presentar cada capítulo en formato de artículo científico, ya que algunos capítulos son versiones preliminares de artículos que van a ser sometidos. Por ello, debo mecionar que existe cierto sobrelape y repetición en las introducciones entre varios de los capítulos que conforman esta tesis.

RESUMEN

Cuando los intereses de machos y hembras difieren, se espera que surja el conflicto sexual. En especies vivíparas polígamas, este tipo de conflicto puede darse en torno a la inversión materna durante el desarrollo embrionario, propiciando la manipulación paterna de la inversión materna. Los machos que sean capaces de incrementar la asignación materna de recursos en su progenie se verán beneficiados si esto vuelve a sus críos más exitosos que los de otros machos, incluso si dicha inversión afecta la inversión futura de las hembras. El conflicto relacionado a efectos antagónicos de genes que controlan la asignación materna de recursos durante el desarrollo embrionario (como el factor de crecimiento tipo insulin 2 -*igf2*-), parece haber llevado a la evolución de la impronta genética en embriones de mamíferos placentarios; sin embargo, poca atención se ha prestado al estudio de este fenómeno en otros organismos vivíparos, tales como reptiles y peces. En los peces, el grado de matrotrofía varía enormemente entre especies, y por lo tanto, también la intensidad del conflicto sexual. Nosotros usamos al pez matrotrófico *Girardinichthys multiradiatus* para buscar evidencia de: 1) manipulación paterna de la asignación materna de recursos durante el desarrollo embrionario, y 2) la evolución de la impronta genética de *igf2* en peces placentados (Goodeidos). Encontramos evidencia que sugiere que 1) los machos manipulan la asignación materna, dando como resultado críos más grandes y pesados en un cruce interpoblacional, y 2) *igf2* podría estar siendo regulado epigenéticamente, dado el patrón de metilación diferencial dependiente del origen del alelo parental que detectamos. Aunque nuestros resultados de expresión alélica de *igf2* fueron inconclusos debido a un tamaño de muestra reducido, la expresión de este gen aparentemente es paterna (monoalélica), y la evolución de su secuencia parece estar siendo moldeada por varias presiones de selección dentro de la familia. Por todo lo anterior, más investigación debe ser dedicada al estudio del efecto que el conflicto sexual tiene en la regulación y evolución de genes en estos peces.

ABSTRACT

Sexual conflict is expected to arise when the interests of the sexes differ. In viviparous polygamous species, this kind of conflict may evolve in relation to maternal investment during embryonic development, leading to a male manipulation of female reproductive investment. Males that can increase maternal investment in their progeny would benefit if this results in more successful offspring than those of their competitors, even if it reduces female potential future reproductive investment. Conflict related to the antagonistic effects of genes controlling maternal allocation during embryonic development (such as *igf2*) has apparently lead to a parent-of-origin gene expression (genomic imprinting) in embryos of therian mammals; however, little attention has been devoted to study this phenomenon in viviparous reptiles and fish. Among fishes, the degree of matrotrophic development varies greatly among species, as does the strength of sexual conflict over maternal allocation. We used the matrotrophic fish *Girardinichthys multiradiatus* to look for evidence of 1) male manipulation of maternal allocation of resources during embryonic development, and 2) the evolution of genomic imprinting of *igf2* in placental fish (Goodeidae). We found evidence suggestive of 1) male manipulation of maternal nutrients that resulted in bigger and heavier offspring in one inter-population cross, and 2) an epigenetic regulation of *igf2* expression, that was inferred from a parent-of-origin methylation pattern in a section of *igf2*. Although our results on allelic expression of *igf2* were inconclusive due to a limited sample size, the expression of this gene is apparently paternal (monoallelic), and the evolution of its sequence appears to have been been shaped by several selective pressures within the family. Thus, more research should be devoted to study the effect of sexual conflict on gene regulation and evolution within the goodeids.

CAPÍTULO 1

INTRODUCCIÓN GENERAL

Los intereses de los individuos raramente coinciden ya que no comparten el mismo genoma. Cuando hay una diferencia en los intereses evolutivos de dos grupos de organismos (i.e. adecuación óptima) se genera el potencial para el conflicto (Parker 1979). Este conflicto puede llevar a una coevolución antagonista, es decir, cuando la aparición de rasgos o atributos que le confieren una ventaja adaptativa a un grupo de organismos, le imponen un costo al otro, y como resultado, el grupo afectado evoluciona contra-adaptaciones para mitigar los efectos negativos que les fueron impuestos. Sin embargo, el grupo "abusivo" siempre puede generar nuevas adaptaciones para seguir teniendo una ventaja sobre el otro (Arnqvist & Rowe 2002).

Por lo anterior, el conflicto tiene el potencial de generar cambios evolutivos (Arnqvist & Rowe 2005), y aunque puede darse a nivel inter-específico, como en el caso de algunas bacterias y sus bacteriófagos (Buckling & Rainey 2002), normalmente se genera entre individuos de la misma especie, como en el caso del conflicto madre-crío (Trivers 1974) o entre hembras y machos (conflicto sexual; Parker 1979; Arnqvist & Rowe 2002). Incluso dentro de un mismo individuo, si los alelos de un mismo locus difieren, entonces es muy probable que haya competencia entre ellos (Arnqvist & Rowe 2005).

Coevolución antagonista y expresión genética

El conflicto entre los sexos ha sido ampliamente documentado en varios grupos de vertebrados e invertebrados (Córdoba-Aguilar 2002; Lessells & McNamara 2012; Macías Garcia & Saldivar Lemus 2012; Okada et al. 2014). En la mayoría de los estudios se habla de una coevolución antagonista entre rasgos fenotípicos de los individuos, sin embargo, también puede ser explicado desde un punto de vista genético.

Cuando la selección favorece diferentes valores de un mismo rasgo fenotípico en machos y hembras, por ejemplo su coloración, o la expresión de un gen (Chippindale et al. 2001), entonces surge lo que se conoce como Conflicto Sexual Intralocus (*Intralocus Sexual Conflict- IASC)*. Cuando la interacción entre machos y hembras lleva a la evolución de adaptaciones y contra-adaptaciones que son codificadas por diferentes loci, entonces se da un Conflicto Sexual Interlocus (*Interlocus Sexual Conflict- IRSC*), que resulta en una coevolución antagonista entre los sexos (Arnqvist & Rowe 2005; Chapman 2006).

El IASC parece ser la fuerza evolutiva que impulsó el origen de la impronta genética (Day & Bonduriansky 2004), un patrón de expresión génica que se da en organismos bialélicos, y que se caracteriza por una expresión monoalélica del gen, donde dependiendo del origen parental de cada alelo, uno se expresa y el otro permanece silenciado. Sin embargo, aunque la impronta genética ha sido categorizada como un IASC, existen interacciones más complejas, producto del conflicto entre los sexos, donde lo que inició como un IASC, puede convertirse en un IRSC, o en lo que Rice & Holland (1997) denominaron como *Interlocus Contest Evolution* (ICE), un proceso de coevolución antagonista en el que interactúan diferentes loci dentro del genoma de una misma especie en un proceso análogo al de la Reina Roja (*Red Queen process*- un proceso de coevolución antagonista en el que depredador y presa constanmente co-evolucionan para alcanzar un cierto tipo de balance-). Tal podría ser el caso del conflicto que en mamíferos ha dirigido la expresión monoalélica de los genes *igf2* (expresado paternamente) e *igf2r* (expresado maternamente).

Igf2 codifica para el factor de crecimiento tipo insulina 2 (Insulin-like growth factor 2 –*igf2*-), una proteína que controla la demanda de nutrientes de los críos y el suministro de nutrientes por parte de la placenta durante el desarrollo embrionario (DeChiara et al. 1990), y cuya sobre expresión podría llevar a las hembras a una inversión excesiva y subóptima de

recursos en una sola camada. Por el contrario, *Igf2r* codifica para uno de los receptores de *igf2* (Insulin-like growth factor receptor- *igf2r-)*, que se encarga de capturar el exceso de *igf2* producido por el alelo paterno y lo lleva a los lisosomas para su posterior degradación (Kornfeld & Mellman 1989; Kornfeld 1992). Bajo este escenario, el conflicto que empezó como un IASC y que llevó a la impronta genética de *igf2* (expresión del alelo paterno y silenciamiento del alelo materno), se convirtió más tarde en un conflicto IRSC, o más específicamente en un ICE, cuando surgió una contra adaptación codificada por otro gen, y que consistió en la evolución de un sitio activo en el receptor manosa-6-fosfato catión-independiente, que fue capaz capturar y degradar el exceso de *igf2* paterno, evitando así una inversión de nutrientes subóptima de las hembras (Haig & Graham 1991).

La evolución de la impronta genética: diferentes teorías

- La Teoría del Conflicto: The Kinship Theory of Genomic Imprinting

La Teoría del Conflicto, también conocida como Teoría del Parentesco, trata de explicar la evolución de la impronta genética basándose en el conflicto que hay entre madres y críos con respecto a la asignación de nutrientes maternos, y que derivó posteriormente en un conflicto entre los alelos heredados vía paterna y vía materna en el embrión (Haig & Westoby 1989). Es decir, en sistemas poligámicos, dado que los críos dentro de una misma camada no están relacionados con sus hermanos de la misma manera en la que están relacionados consigo mismos, esto puede llevar a los críos a manipular la cantidad de recursos que reciben de su madre, y esta sobre inversión puede no ser la óptima para ella, llevando así a un conflicto madre-crío con respecto a la asignación materna de recursos. Particularmente, ya que en sistemas poligámicos las hembras tienen camadas de diferentes machos, e incluso los críos de una misma camada pueden ser engendrados por diferentes padres, aunque las hembras se ven beneficiadas al procrear individuos competitivos que son capaces de sobrevivir y reproducirse (eso incrementa su propia adecuación), para los

embriones es benéfico recibir una cantidad mayor de nutrientes que la que reciben sus hermanos o medios hermanos, aunque ello pueda afectar la reproducción futura de la madre. Este tipo de conflicto parece haber llevado a una coevolución antagonista dentro del genoma del crío, entre los alelos derivados maternamente y paternamente de los genes que influyen en la asignación de recursos maternos durante el desarrollo embrionario, cuyas adaptaciones y contra adaptaciones han resultado en una expresión monoalélica de algunos genes (Moore & Haig 1991).

Esta teoría propuesta por Haig (2000), plantea que en sistemas poliándricos en el genoma paterno se va a favorecer la evolución de adaptaciones (i.e. sobre expresión de genes responsables de la comunicación alimentaria madre-crío como el *igf2*) que favorezcan un incremento de la extracción de recursos maternos, lo cual se va a ver reflejado en un incremento de la adecuación de los críos de ese padre, sin importar que eso traiga consigo un decremento en la adecuación de los futuros críos de la madre. Por el contrario, se espera que en el genoma materno evolucionen adaptaciones que promuevan una distribución equitativa de recursos entre las diferentes camadas a lo largo de la vida reproductiva de la hembra, independientemente del padre de los críos, como por ejemplo silenciando su alelo de *igf2* en el crío para lograr un balance en la producción de IGF2.

Este tipo de conflicto, a pesar de que refleja una diferencia en los intereses entre alelos paternos y maternos presente en el crío, en el momento en el que afecta la adecuación de la madre (costo) y favorece la adecuación del padre (beneficios), puede extrapolar a un conflicto entre los sexos, a pesar de que el “campo de batalla” sea a nivel genético entre los alelos parentales en el crío.

- La Hipótesis de Coadaptación

Otra de las teorías fuertemente apoyadas que trata de explicar la evolución de la impronta genética es la de la coadaptación. Esta hipótesis establece que en los embriones, la selección natural favorece que la expresión genética de la mayoría de los genes impresos sea materna, ya que eso permite una mejor integración adaptativa del genoma materno y del crío, y por lo tanto, un incremento en la adecuación del crío (Wolf & Hager 2006). De acuerdo a los autores de esta teoría, la impronta genética regula coadaptativamente el desarrollo embrionario de mamíferos, el cuidado materno y la conducta reproductiva (Curley *et al*. 2004; Keverne & Curley 2008).

Uno de los ejemplos clásicos que respalda esta hipótesis, a pesar de tratarse de un gen que se expresa paternamente, es el del gen Peg3, el cual está impreso en la placenta, y en el hipotálamo del feto y de la madre (Renfree *et al*. 2009). Peg3 se expresa paternamente en el cerebro y en la placenta, y es esencial para una conducta lactante adecuada en el crío (Curley *et al*. 2005). La expresión de Peg3 también ha sido asociada al tamaño de la placenta, al crecimiento embrionario, al crecimiento post natal, a la edad de destete, al comienzo de la pubertad, al cuidado maternal, a la ingesta materna de recursos durante la gestación, y a una bajada de la leche disminuida. Este gen se expresa paternamente, y por lo tanto cuando la copia paterna de *Peg3* es interrumpida en la madre o en los críos, la conducta lactante, el cuidado maternal y el desarrollo embrionario de los críos son afectados (Curley *et al*. 2004).

De acuerdo a Curley y colaboradores (2004), la expresión de estos genes es coadaptativa, ya que los críos portadores de esos genes no sólo serán bien provistos por sus madres, también tendrán la predisposición ser buenos proveedores de recursos y cuidados para su progenie. Este tipo de ventaja adaptativa genera una presión de selección que fomenta la fijación y esparcimiento de esa característica en la población y/o especie.

De acuerdo a Wolf & Hager (2006), esta teoría sostiene que la mayoría de los genes que incrementan la adecuación de los crío y que involucran una interacción madre-crío serán expresados maternamente; sin embargo, aunque la teoría de la coadaptación encaja en una gran cantidad de genes impresos, no descarta que la impronta genética haya evolucionado en diferentes loci como producto de diferentes presiones selectivas, ya que no puede explicar la impronta de genes tales como *igf2* e *igf2r*.

Igf2 e *igf2r*

El factor de crecimiento tipo insulina 2 (*igf2*) es un gen que codifica para una proteína muy semejante a la pro-insulina (Wood et al. 2005). IGF2 juega un papel muy importante durante el desarrollo embrionario, ya que tiene efectos en el crecimiento y en la diferenciación celular, y además es esencial para una adecuada transferencia de nutrientes durante el desarrollo embrionario (DeChiara et al. 1991; Werner et al. 1994)(Werner et al. 1994; DeChiara et al. 1991).

Esta proteína tiene dos receptores. IGF1R, que a pesar de ser un receptor más específico para el factor de crecimiento tipo insulina 1 (IGF1), también captura a IGF2, para un adecuado crecimiento y desarrollo de los individuos (Germain-Lee et al. 1992; Le Roith 1997). Por otro lado está IGF2R, que se encarga de capturar el exceso de IGF2 circulante para llevarlo posteriormente a los lisosomas donde será degradado (Morgan et al. 1987). IGF2R es también conocido como el receptor catión-independiente manosa-6-fosfato, y aunque su función principal es la captura de manosa-6-fosfato, también tiene un sitio activo que evolucionó posteriormente, y que reconoce y captura a IGF2 (Kornfeld & Mellman 1989).

Como mencioné anteriormente, i*gf2* e *igf2r* tienen patrones de metilación inversos. Mientras la producción de IGF2 es exclusivamente paterna, IGF2R es producido sólo mediante la expresión del alelo materno (DeChiara et al. 1990; Ludwig et al. 1996); sin

embargo, la expresión monoalélica de estos genes que aparentemente alcanzó un equilibrio durante dicha coevolución antagonista, es necesaria para producir descendencia viable. Cuando el alelo paterno de *igf2* es interrumpido en ratones, la descendencia es de 60% del tamaño del fenotipo silvestre (DeChiara et al. 1990), mientras que si el alelo materno de IGF2 es interrumpido en ratones mutantes, estos tienen un incremento de 135% del tamaño normal al nacer (Ludwig et al. 1996).

Viviparidad, poliginia y conflicto

La reproducción es costosa, ya que los individuos deben de invertir de diferentes maneras (en la producción de gametos, en nutrientes durante el desarrollo embrionario, cuidado parental) en cada evento reproductivo (Trivers 1972; Clutton-Brock 1991). Aunque ambos padres comparten el mismo interés en la supervivencia de los críos, dependiendo del sistema de apareamiento pueden pagar costos sumamente diferentes para lograrlo (Gross & Sargent 1985). Principalmente en sistemas polígamos, para los padres siempre será benéfico hacer al otro invertir más, ya que el incremento en el cuidado por parte de un sexo permitiría una disminución en el cuidado por parte del otro, o favorecería un incremento en la calidad de los críos.

Teóricamente, en sistemas monógamos machos y hembras comparten los mismos intereses, ya que el bienestar de ambos es necesario para su éxito reproductivo y la supervivencia de sus diferentes camadas; sin embargo, cuando existe una inversión parental desigual en la reproducción, que puede empezar por la anisogamia, se crea una diferencia en los intereses de los sexos, que es capaz de crear un conflicto (Chapman 2006). En un sistema polígamo donde además los individuos tienen una nueva pareja en cada evento reproductivo, el bienestar de la pareja deja de ser importante, y sólo la supervivencia y la producción de críos de buena calidad es el factor trascendente. Esto

puede llevar a la evolución de estrategias en un sexo, que favorezcan que el miembro del otro sexo invierta más de su óptimo en la camada que comparten.

En el caso de los animales vivíparos, donde sólo las hembras proveen recursos a los críos durante el desarrollo embrionario, y donde hay una comunicación fisiológica y a veces una conexión física (placentas) madre-crío, se crea el escenario perfecto para una coevolución antagonista entre machos y hembras con respecto a la inversión materna durante el desarrollo embrionario. Los machos (o los alelos paternos en el crío) capaces de hacer que sus críos (portadores) extraigan una mayor cantidad de nutrientes, se verán favorecidos al tener críos con una mayor adecuación; sin embargo, adaptaciones que favorezcan dicha capacidad en los críos, pueden desencadenar un proceso coevolutivo antagonista (entre machos y hembras, o entre alelos paternos y maternos en el crío) al manipular a la madre para que invierta nutrientes de más, como el que se propone dio origen a la impronta genética de los genes *igf2* e *igf2r* (Haig 2004).

Wilkins y Haig (2003) propusieron que la evolución de la impronta genética como producto del conflicto entre alelos parentales en el crío (o desde nuestro punto de vista, el conflicto sexual) sólo será favorecida en grupos donde: i) las hembras tengan críos de más de un padre (pologamia), ii) los costos de crecimiento de los críos caigan preferentemente sobre la madre (viviparidad), y iii) los genes que son expresados en el descendiente puedan influir en la distribución materna de los recursos. Este proceso se ha documentado en mamíferos (Constância et al. 2002; O'Neill et al. 2000) y en plantas (Scott et al. 1998; Alleman & Doctor 2000), y es de esperar que ocurra en otros taxa vivíparos tales como los goodeinos, una subfamilia de peces dulceacuícolas endémicos de México Central, que destacan por sus adaptaciones en torno a la viviparidad y a la matrotrofía, un tipo de nutrición embrionaria en el cual la madre provee de nutrientes a los embriones durante la gestación (Wourms 1981). Este modo de nutrición embrionaria difiere de la lecitotrofía en

que la cantidad de nutrientes que la madre le da a sus críos es notoriamente mayor, ya que los organismos vivíparos lecitotróficos dependen de los nutrientes que la madre puso en la yema para alimentarse (Wourms et al. 1991), por lo que la asignación materna de nutrientes es fija y no se puede modificar después de la formación del cascarón.

Especie de estudio

El Pez Amarillo, *Girardinychthys multiradiatus*, es un pez vivíparo matrotrófico que pertenece a la familia Goodeidae (subfamilia Goodeinae). Los goodeinos se distribuyen en cuerpos de agua dulce de México Central y contienen aproximadamente 36 especies distribuidas en 16 géneros (Webb et al. 2004).

Como mencioné anteriormente, estos peces son altamente matrotróficos, es decir, el huevo contiene un minúsculo suministro de vitelo y la nutrición del embrión se da vía materna a lo largo de la gestación. Además, han desarrollado lo que se conoce como placenta trofotenial, una estructura análoga a la placenta de mamíferos, que permite al embrión recibir lipoproteínas y aminoácidos maternos y aumentar su tamaño hasta 38,700% dentro del lumen ovárico de la madre (*Zoogoneticus quitzeoensis*; Wourms et al. 1988; Hollenberg & Wourms 1995). Esto es posible gracias al intercambio que se presenta entre el epitelio trofotenial (tejido embrionario) y el epitelio interno del ovario (tejido de la madre; Lombardi & Wourms 1985a).

El conflicto entre sexos en relación con el apareamiento ha sido demostrado en esta familia (Macías Garcia & Ramirez 2005) y podría estar promoviendo la especiación (Ritchie et al. 2007).

Una de las especies mejor estudiadas, y en donde podría haber evolucionado el conflicto sexual dadas sus características en torno a la reproducción es *G. multiradiatus.* Las poblaciones de este pez han divergido fenotípica- (González Zuarth & Macías Garcia

2006) y genéticamente (Macías Garcia et al. 2012), y además se ha demostrado la ocurrencia de paternidad múltiple (Macías-Garcia & Saborío 2004).

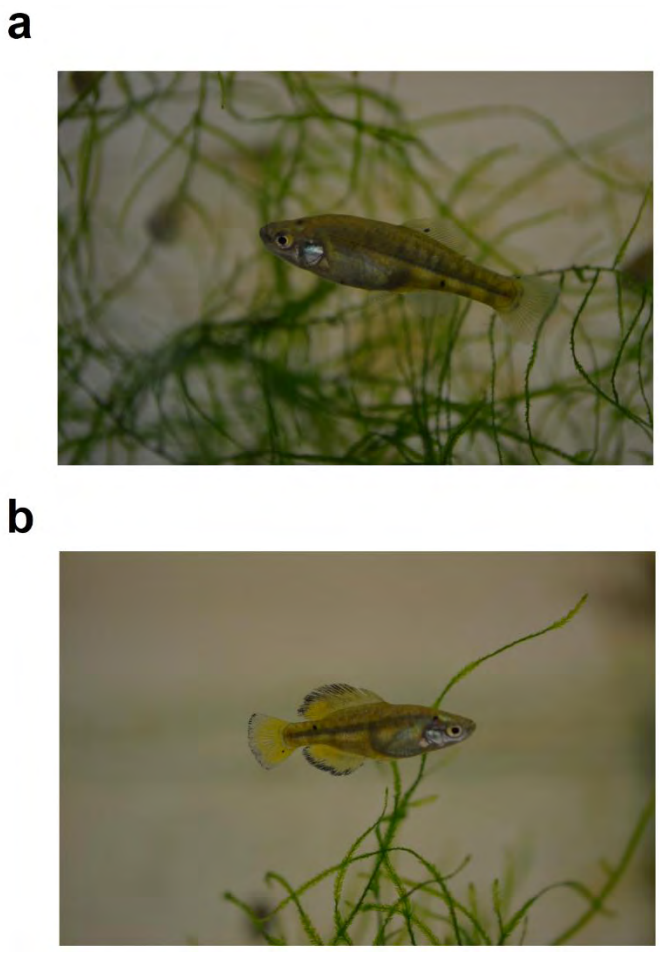


Figura 1. Hembra (a) y macho (b) de *G. multiradiatus*.

Girardinichthys multiradiatus es una especie con dimorfismo sexual, ya que las aletas caudal y anal de los machos son más grandes y más coloridas que las de las hembras (Figura 1). Este pez habita en lugares altos y fríos de las cuencas del Lerma, Balsas y Pánuco (Figura 2). Se separó de su especie hermana *Girardinichthys viviparus* hace aproximadamente 7.9 millones de años (Gesundheit & Macias-Garcia 2005), posiblemente por un evento de vicarianza- alopatría (Webb et al. 2004), y dado que su

distribución se extiende a lo largo de varios kilómetros existen diversas poblaciones de este pez que se enfrentan a condiciones ambientales distintas y que pueden llegar a presentar entre sí distancias geográficas y genéticas notables, como es el caso de las poblaciones de San Matías el Grande en Michoacán, y de Zempoala en el Estado de México.

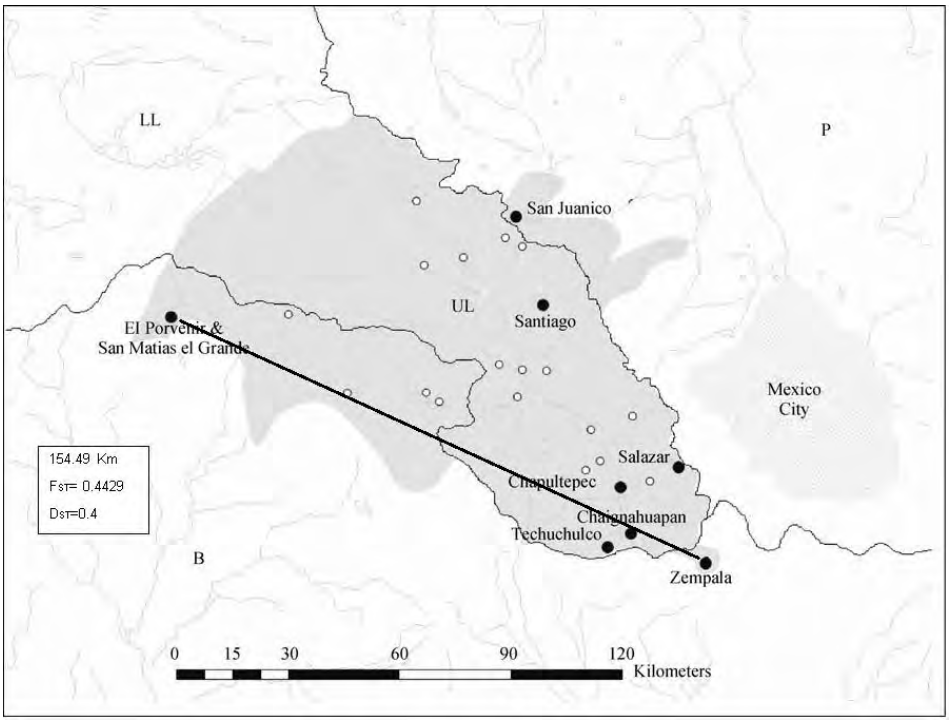


Figura 2. Mapa de la distribución de *G. multiradiatus*, y de la distancia genética entre dos de las poblaciones, escala 1:1 000 000. En el recuadro se muestra la distancia en kilómetros entre las dos localidades más lejanas, el índice de fijación de Wright (FST), y el Promedio de Diversidad Genética entre Poblaciones de Nei (DST). Las líneas negras dividen las cuencas Panuco (P), Balsas (B) y Lerma (Lerma superior, UL; Lerma inferior, LL). Datos obtenidos y mapa modificado de Macías Garcia et al. 2012.

Justificación

En mamíferos con un sistema de apareamiento polígamo, la evolución de la viviparidad abrió el camino a la ocurrencia de un conflicto entre machos y hembras con respecto a la

asignación materna de recursos que es óptima para cada sexo. Este conflicto posiblemente dio origen a la evolución de la impronta genética de algunos genes relacionados a la transferencia de nutrientes. Ya que los goodeinos están conformados por especies polígamas (lo cual intensifica el conflicto sexual), exhiben placentotrofía (lo cual permite una transmisión eficiente de recursos madre-crío), y tienen expresión de *igf2* durante el desarrollo embrionario (lo que permite la manipulación de recursos maternos), el propósito de esta tesis fue abordar varios grupos de preguntas (distribuidos en los diferentes capítulos) en torno a la viviparidad, el conflicto, y la evolución de la impronta genética (más específicamente de *igf2)* utilizando como modelo al pez matrotrófico y polígamo *Girardinichthys multiradiatus*.

Capítulo 2: Conflicto e impronta genética en vertebrados vivíparos.

El origen y la evolución de la viviparidad permitió un aumento en la supervivencia de los críos; sin embargo, los costos para las hembras no sólo estuvieron limitados a un gasto energético más alto (relacionado a la motilidad) y a una supervivencia menor (debido a la capacidad reducida para huir de los depredadores). Las características que permitieron un mejor y más eficiente aprovisionamiento de recursos, también crearon el campo de batalla para que diferentes tipos de conflicto, con respecto a la asignación materna de recursos, tomaran lugar. Por ello, primero hicimos una revisión que respondiera preguntas como ¿Qué fuerzas evolutivas promovieron la evolución de la viviparidad? ¿Qué tipo de conflicto trajo consigo la evolución de la viviparidad y la placentotrofía y en qué tipo de sistema de apareamiento se puede ver favorecida la manipulación de recursos maternos? Si la impronta genética evolucionó como resultado del conflicto entre alelos maternos y paternos en el crío con respecto a la asignación materna de recursos ¿En qué otros grupos de vertebrados es posible que haya evolucionado o pueda evolucionar?

Capítulo 3: ¿Qué es la impronta genética? Teorías, fundamentos y mecanismos.

En este capítulo hicimos una revisión bibliográfica que nos permitiera responder preguntas teóricas como ¿Qué es la impronta genética? ¿Cuál es su base molecular? ¿Cuáles son las características de los genes impresos? ¿Qué fuerzas evolutivas favorecieron el surgimiento y evolución de la impronta genética en genes como *igf2*?

Capítulo 4: ¿Efectos asimétricos paternos en la talla de los críos revelan manipulación paterna de la inversión materna?

Los goodeinos son peces vivíparos (placentados) y poligámicos. Estas características, que son consideradas como el principal motor para el surgimiento de un conflicto entre machos y hembras con respecto a la inversión parental en los críos, nos hicieron sospechar que en los machos se han favorecido adaptaciones que han permitido incrementar la asignación materna de recursos durante el desarrollo embrionario. Por ello, en este capítulo tratamos de responder las interrogantes de si los machos de *Girardinichthys multiradiatus* son capaces de incrementar la asignación materna de recursos durante el desarrollo embrionario (reflejado como un incremento en peso y/o talla de los críos), y si este tipo de manipulación de recursos ha llevado a una coevolución antagonista entre machos y hembras.

Capítulo 5: El *factor de crecimiento tipo insulina 2* de los Goodeidos

Nuestros resultados del capítulo 4 sugirieron que los machos de *G. multiradiatus* son capaces de incrementar la cantidad de recursos maternos durante el desarrollo embrionario. *Igf2* ha sido relacionado a este tipo de conflicto en mamíferos marsupiales y euterios, por lo cual nos dimos a la tarea de describir a este gen dentro de la subfamilia Goodeinae, guiándonos por las preguntas: ¿Qué características tiene el *igf2* de los

goodeinos? ¿Qué tanta variación existe en la secuencia dentro de las poblaciones de *G. multiradiatus* y dentro de la familia?

Capítulo 6: ¿*Igf2* se expresa en una manera dependiente del origen parental en un pez vivíparo matrotrófico?

Una vez que describimos el gen, y que notamos que al parecer diferentes fuerzas evolutivas están moldeando su secuencia, indagamos si el patrón de expresión de *igf2* estaba ligado a los resultados fenotípicos que obtuvimos en el capítulo 4. Por ello, procuramos responder las siguientes interrogantes: ¿La expresión del *igf2* en *G. multiradiatus* está ligada y/o ha sido dirigida por una coevolución antagonista entre machos y hembras con respecto a la asignación materna de recursos? Y más específicamente ¿La impronta genética de *igf2* ha evolucionado en este grupo de peces vivíparos como resultado del conflicto sexual?

OBJETIVO GENERAL

Determinar si en peces vivíparos de la familia Goodeidae existe un conflicto entre machos y hembras con respecto a la asignación materna de recursos durante el desarrollo embrionario. De ser así, determinar si este conflicto ha favorecido la evolución de la impronta genética en genes a cargo de la comunicación alimentaria entre madres y críos (insulin-like growth factor 2- *igf2*-).

OBJETIVOS PARTICULARES

- Realizar una revisión extensiva de la literatura que existe actualmente en torno a la viviparidad, a los sistemas de apareamiento y a la impronta genética, para poder hacer predicciones sobre la evolución de la impronta genética en otros grupos de vertebrados.
- Determinar fenotípicamente si los machos de *Girardinichthys multiradiatus* son capaces de manipular/incrementar la inversión materna de recursos durante el desarrollo embrionario.
- Describir la variación intra- e inter-específica de las secuencias (genómicas y codificantes) de *igf2* en los goodeinos.
- Determinar si *igf2* está impreso (se expresa monoalélicamente) en esta familia de peces vivíparos.

Bibliografía

Alleman, M. & Doctor, J., 2000. Genomic imprinting in plants: observations and evolutionary implications. *Plant molecular biology*, 43, pp.147–161.

Arnqvist, G. & Rowe, L., 2002. Antagonistic coevolution between the sexes in a group of insects. *Nature*, 415, pp.787–789.

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

Buckling, A. & Rainey, P.B.., 2002. Antagonistic coevolution between a bacterium and a bacteriophage. *Proceedings of the Royal Society B: Biological Sciences*, 269(1494), pp.931–936.

Chapman, T., 2006. Evolutionary Conflicts of Interest between Males and Females. *Current Biology*, 16(17), pp.744–754.

Chippindale, A.K., Gibson, J.R. & Rice, W.R., 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America*, 98(4), pp.1671–1675.

Clutton-Brock, T.H., 1991. *The Evolution of Parental Care* J. R. Krebs & T. H. Clutton-Brock, eds., New Jersey: Princeton University Press.

Constância, M. et al., 2002. Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature*, 417(6892), pp.945–948.

Córdoba-Aguilar, a, 2002. Sensory trap as the mechanism of sexual selection in a damselfly genitalic trait (Insecta: Calopterygidae). *The American naturalist*, 160(5), pp.594–601.

Curley, J.P. et al., 2004. Coadaptation in mother and infant regulated by a paternally expressed imprinted gene. *Proceedings. Biological sciences / The Royal Society*, 271(1545), pp.1303–1309.

Curley, J.P. et al., 2005. Increased body fat in mice with a targeted mutation of the paternally expressed imprinted gene Peg3. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 19(4), pp.1302–1304.

Day, T. & Bonduriansky, R., 2004. Intralocus Sexual Conflict Can Drive the Evolution of Genomic Imprinting. *Genetics*, 167(4), pp.1537–1546.

DeChiara, T.M., Efstratiadis, A. & Robertson, E.J., 1990. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature*, 345, pp.78–80.

DeChiara, T.M., Robertson, E.J. & Efstratiadis, A., 1991. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell*, 64(4), pp.849–859.

Germain-Lee, E.L. et al., 1992. Expression of a type I insulin-like growth factor receptor with low affinity for insulin-like growth factor II. *The Biochemical journal*, 281, pp.413–417.

Gesundheit, P. & Macias-Garcia, C., 2005. Biogeografía cladística de la familia Goodeidae (Cyprinodontiformes). In J. Llorente-Bousquets & J. Morrone, eds. *Regionalización biogeográfica en Iberoamérica y tópicos afines*. Ciudad de México: Facultad de Ciencias, UNAM, pp. 319–338.

González Zuarth, C. & Macías Garcia, C., 2006. Phenotypic differentiation and pre-mating isolation between allopatric populations of Girardinichthys multiradiatus. *Proceedings. Biological sciences / The Royal Society*, 273(1584), pp.301–7.

Gross, M.T. & Sargent, R.C., 1985. The Evolution of Male and Female Parental Care in Fishes. *American Zoologist*, 25(3), pp.807–822.

Haig, D., 2004. Genomic imprinting and kinship: how good is the evidence? *Annual review of genetics*, 38, pp.553–585.

Haig, D., 2000. The Kinship Theory of Genomic Imprinting. *Annual Review of Ecology and Systematics*, (34), pp.9–32.

Haig, D. & Graham, C., 1991. Genomic Imprinting and the Strange Case of the Insulin-like Growth Factor II Receptor. *Cell*, 64, pp.1045–1046.

Haig, D. & Westoby, M., 1989. Parent-Specific Gene Expression and the Triploid Endosperm. *The American Naturalist*, 134(1), p.147.
Hollenberg, F. & Wourms, J.P., 1995. Embryonic growth and maternal nutrient sources in goodeid fishes (Teleostei: Cyprinodontiformes). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 271(5), pp.379–394.
Keverne, E.B. & Curley, J.P., 2008. Epigenetics, brain evolution and behaviour. *Frontiers in Neuroendocrinology*, 29(3), pp.398–412.
Kornfeld, S., 1992. Structure and function of the mannose 6-phosphate insulin-like growth factor-II receptors. *Annu. Rev. Biochem*, 61, pp.307–330.
Kornfeld, S. & Mellman, I., 1989. The biogenesis of lysosomes. *Annu. Rev. Cell Biol.*, 5, pp.483–525.
Lessells, C.M. & McNamara, J.M., 2012. Sexual conflict over parental investment in repeated bouts: negotiation reduces overall care. *Proceedings of the Royal Society B: Biological Sciences*, 279(1733), pp.1506–14.
Lombardi, J. & Wourms, J.P., 1985. The trophotaenial placenta of a viviparous goodeid fish. I: Ultrastructure of the Internal Ovarian Epithelium, the Maternal Component. *Journal of molecular biology*, 184, pp.277–292.
Ludwig, T. et al., 1996. Mouse Mutants Lacking the Type 2 IGF Receptor (IGF2R) Are Rescued from Perinatal Lethality …. *Developmental Biology*, 535(182), pp.517–535.
Macías-Garcia, C. & Saborío, E., 2004. Sperm competition in a viviparous fish. *Environmental Biology of Fishes*, 70(3), pp.211–217.
Macías Garcia, C. et al., 2012. Variation in sexual dimorphism and assortative mating do not predict genetic divergence in the sexually dimorphic Goodeid fish Girardinichthys multiradiatus. *Current Zoology*, 58(3), pp.440–452.
Macías Garcia, C. & Ramirez, E., 2005. Evidence that sensory traps can evolve into honest signals. *Nature*, 434(7032), pp.501–505.
Macias Garcia, C. & Saldivar Lemus, Y., 2012. Foraging costs drive female resistance to a sensory trap. *Proceedings of the Royal Society B: Biological Sciences*, 279(1736), pp.2262–2268.
Moore, T. & Haig, D., 1991. Genomic imprinting in mammalian development: a parental tug-of-war. *Trends in Genetics*, 7(2), pp.45–49.
Morgan, D.O. et al., 1987. Insulin-like growth factor II receptor as a multifunctional binding protein. *Nature*, 329(6137), pp.301–7.
O'Neill, M.J. et al., 2000. Allelic expression of IGF2 in marsupials and birds. *Development genes and evolution*, 210, pp.18–20.
Okada, K. et al., 2014. Sexual conflict over mating in Gnatocerus cornutus ? Females prefer lovers not fighters. *Proceedings. Biological sciences / The Royal Society*, 281(May), p.20140281.
Parker, G.A., 1979. Sexual selection and sexual conflict. In M. Blum & N. Blum, eds. *Sexual Selection and Reproductive Competition in Insects*. New York: New York Academy Press, pp. 123–166.
Renfree, M.B. et al., 2009. Evolution of genomic imprinting: insights from marsupials and monotremes. *Annual review of genomics and human genetics*, 10, pp.241–262.
Rice, W.R. & Holland, B., 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behav Ecol and Sociobiol*, 41, pp.1–10.
Ritchie, M.G. et al., 2007. Sex and differentiation: Population genetic divergence and sexual dimorphism in Mexican goodeid fish. *Journal of Evolutionary Biology*, 20, pp.2048–2055.
Le Roith, D., 1997. Insulin-like growth factors. *New England Journal of Medicine*, 336(9), pp.633–640.

Scott, R.J. et al., 1998. Parent-of-origin effects on seed development in Arabidopsis thaliana. *Development*, 125(17), pp.3329–3341.
Trivers, R.L., 1974. Parent-Offspring Conflict. *American Zoologist*, 14(1), pp.249–264.
Trivers, R.L., 1972. Parental Investment and Sexual Selection. In *Selection & the Descent of Man, , New York*. New York: Aldine de Gruyter, pp. 134–179.
Webb, S.A. et al., 2004. Molecular phylogeny of the livebearing Goodeidae (Cyprinodontiformes). *Molecular Phylogenetics and Evolution*, 30(3), pp.527–544.
Werner, H. et al., 1994. Molecular and cellular aspects of the insulin-like growth factor action. *Vitamins and Hormones*, 48, pp.1–58.
Wilkins, J.F. & Haig, D., 2003. What good is genomic imprinting: the function of parent-specific gene expression. *Nature Reviews Genetics*, 4(5), pp.359–368.
Wolf, J.B. & Hager, R., 2006. A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLoS Biology*, 4(12), pp.2238–2243.
Wood, A.W., Duan, C. & Bern, H. a., 2005. Insulin-like growth factor signaling in fish. *International Review of Cytology*, 243, pp.215–285.
Wourms, J.P., 1981. Viviparity: The maternal-fetal relationship in fishes. *Integrative and Comparative Biology*, 21(2), pp.473–515.
Wourms, J.P., Atz, J.W. & Stribling, M.D., 1991. Viviparity and the maternal-embryonic relationship in the coelacanth Latimeria chalumnae. *Environmental Biology of Fishes*, 32(1–4), pp.225–248.
Wourms, J.P., Grove, B. & Lombardi, J., 1988. The maternal-embryonic relationship in viviparous fishes. In W. Hoar & D. Randall, eds. *Fish Physiology*. New York: Academic Press, pp. 1–134.

CHAPTER 2

The evolution of viviparity increased offspring survival; however, it set the arena for a conflict between mother and offspring, males and females, and parental genomes within the offspring regarding the maternal allocation of resources during embryonic development.

Conflicto y la evolución de la viviparidad en vertebrados

Conflict and the evolution of viviparity in vertebrates

Yolitzi Saldívar Lemus[1] & Constantino Macías Garcia[1]*

[1] Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado postal 70-275, Ciudad de México, 04510, México

*Corresponding author. Instituto de Ecología, Universidad Nacional Autónoma de México, A. P. 70-275, C. P. 04510, Ciudad de México, México. E-mail: maciasg@unam.mx. Fax: +5255 56161976

Manuscript

Abstract.-

Viviparity has evolved gradually from oviparity approximately 142 times among vertebrates. Different theories have tried to explain the evolution of viviparity in different taxa; however, none of them is applicable to all the viviparous vertebrates, since each group has lived under different environmental conditions and has faced different selective pressures. We compiled information and developed a panorama that includes environmental, conflicting and coadaptative selective pressures acting together (although not at the same time nor in all taxa), which we consider originated the different forms of viviparity, and the diverse maternal-foetal adaptations for nutrient transfer and consumption within each group. Although the evolution of viviparity seems to be adaptive for both, parents and offspring, conflicting interests among mothers, fathers and offspring regarding the allocation of maternal resources may lead to an antagonistic coevolution of paternal (or embryonic) and maternal adaptations to increase and restrain the maternal supply of nutrients respectively. At a genomic level, this kind of adaptations may have effects on gene expression as those that favoured the evolution of genomic imprinting in Therian mammals. Although this epigenetic phenomenon is thought to be exclusive of placental mammals, more research should be devoted to viviparous squamates and fish, whose attributes are potential enhancers of conflict.

Keywords: vertebrates, genomic imprinting, matrotrophy, lecithotrophy, placenta

Introduction

Even in its simplest forms, reproduction is costly, since either self-dividing or producing and releasing gametes use resources (Stearns 1989; Blacher et al. 2017; Tarwater & Arcese 2017). These costs are referred to as reproductive investment, because they are incurred in order to gain fitness benefits through reproduction. Investment in individual offspring can be made in one step, as when females produce yolk-provisioned eggs, or may be deferred through a gestation period via embryo-maternal (or paternal in a few cases) communication. Moreover, parents frequently continue investing in their offspring after hatching or birth, for instance by providing food, grooming or defence. However, although producing one descendant may convey the same reproductive benefit to both parents, the net fitness gain accrued by each parent may not be the same if their respective parental contributions are not equal (Gross & Sargent 1985). For instance, withholding parental investment can be advantageous if the partner can provide enough resources for the offspring to reach independence, because the same fitness will be accrued with less investment. This is a type of sexual conflict, which more generally occurs when the evolutionary interest of males and females differ, or when their optima cannot simultaneously be realised (Trivers 1972). Sexual conflict can arise in relation to courtship, current or future mating decisions (Parker 1974; Smuts & Smuts 1993; Magurran & Seghers 2008; Arnqvist & Rowe 2002), as well as over parental investment (Trivers 1972), and it may lead to the evolution of traits (e.g. behaviours or proteins production = gene expression) that are beneficial to one sex, but that impose a cost to the other (Parker 1974). Such antagonistic coevolution resembles an evolutionary arms race, where adaptations to bring the interaction closer to the optimum value for one sex are met by counter-adaptations towards the optimum value for the other sex.

For a parent it is typically beneficial to make its partner to invest more than itself in their common offspring (e.g. Osorno & Székely 2004; McNamara et al. 2003), because it

enables a reduction in the amount of care it provides. Thus, sexually antagonistic coevolution is expected to give rise to attributes that induce partners to increase the amount of care they provide, and on the partners of attributes in charge of resisting such manipulation (reviewed in Chapman et al. 2003). Although demonstrations of antagonistic coevolution often deal with phenotypic traits such as those used to induce mating (Arnqvist & Rowe 2002; Macías Garcia & Ramirez 2005), it has also been reported in relation to traits that influence provisioning to developing offspring, such as the augmented maternal investment by birds exposed to attractive male traits (e.g. Burley 1981; Gil et al. 1999). Sexual conflict can occur at the simpler but very transcendental level of the expression of genes in charge of regulating nutrient transfer during offspring development (Moore & Haig 1991) such as the insulin-like growth factor 2 (*igf2*) and its receptor (*igf2r*; Haig and Graham, 1991) or it might also be related to activation of signalling pathways of proteins regulating nutrient intake. The evolution of adaptations and counter-adaptations of genes that control nutrient transfer during embryonic development is indeed one of the explanations for the evolution of genomic imprinting, by which parental alleles are monoallelically expressed in a parent-of-origin manner (Haig & Westoby 1989).

Viviparity

Animal viviparity has evolved independently over 160 times, including 142 instances of convergent evolution amongst vertebrates (Blackburn 1999). Given the diversity of conditions currently experienced by viviparous taxa, it is not clear which selective force, or forces, promoted viviparity in the first place. This mode of reproduction confers a variety of real or suspected fitness benefits, several of which have been proposed as the primary forces driving its evolution (e.g. aiding embryo development through thermoregulation, or reducing egg predation risk; Blackburn 1999), yet each tends to be relevant only in some of the viviparous taxa, thus we lack a global theory for the evolution of animal viviparity. Here

we attempt to fill this gap in relation to vertebrate viviparity, although we hope that our proposal can be applied also to viviparity in other animal taxa.

The transition from oviparity to viviparity must have been gradual (Blackbun 1992), and would have been possible thanks to the evolution of 1) egg retention (Blackburn & Evans 1986) and internal fertilization, then 2) internal gestation within (typically) the female reproductive tract, where embryos are nourished only with nutrients contained in the vitellum (yolk) of the ovum (lecithotrophy; Blackburn 2000). Subsequently, 3) gradual lengthening of developing time is matched by increasing supplementation of nutrients released by the mother into her reproductive tract (incipient matrotrophy), culminating in what we know as 4) matrotrophy or matrotrophic viviparity (Blackburn 2000), where instead of yolk, nutrients are regularly provided by the mother in the form of oviductal secretions or through placental organs. Thus, from an egg-laying ancestor, females would have become able to give birth to bigger and fully developed offspring, potentially capable of self-sufficiency (see supplementary materials). The above route, however, has not been followed by all viviparous taxa (Blackburn 1992), which highlights the need for a theoretical framework that may accommodate also more divergent paths towards matrotrophic viviparity.

Viviparity involves several costs, yet it has evolved repeatedly because these are vastly outweighed by the benefits that it generates. For the mother, bearing fertilised eggs is energetically demanding, it increases the risk of predation -with the concomitant loss of the entire brood-, and reduces the number of offspring that can be produced in any single brood (Blackburn 1999). But it also means greater offspring survival through constant protection of the embryos, adjustable predator avoidance, and, in matrotrophic systems, continuous nutrient supply and adjustable maternal allocation -which minimise the time during which females are encumbered- (Wourms & Lombardi 1992; Blackburn 1999).

Theories of the evolution of matrotrophy and placentation

Both mutually adaptive and conflict hypotheses have been proposed to explain the evolution of matrotrophy and the origin and evolution of placentae. Viviparity *sensu lato*, is a way of reproduction in which eggs are fertilized and retained inside the female (or in some cases the male) where they develop, being afterwards released as completely developed -and sometimes independent- offspring (Patzner 2008).

The driving forces behind the transition from lecithotrophy to matrotrophy are thought to be ecological, such as the locomotor costs imposed by a prolonged period bearing yolked eggs (Blackburn 1999; Ghalambor et al. 2004; Pollux et al. 2009), or the possibility of modifying temporarily the allocation of resources to embryos depending on resource availability (Trexler 1997; Blackburn 1999; Trexler & DeAngelis 2003)(Blackburn 1999; Trexler 1997), without compromising brood or offspring size. Alternatively, we shall argue that the transition may have been driven by post-zygotic genetic conflicts, either among mother and offspring, between partners, or among siblings. The proposal is that genetic weapons, such as embryonic or paternal genes capable of manipulating the maternal physiology, could have promoted the evolution of protracted nutrient transfer. Thus, rather than being consequence of diverse ecological factors, the differences in maternal provisioning among viviparous animals would have been an outcome of the way intra-family genetic conflict over the allocation of maternal resources evolves in different taxa -and may have promoted lineage divergence (Zeh & Zeh 2000)-. Indeed, while the basic set of hormones involved in the regulation of reproductive processes is highly conserved among vertebrates, their sources, functions, and targets differ among taxa, and Crespi & Semeniuk (2004) have proposed that such variation may reflect a long evolutionary history of maternal–foetal antagonistic coevolution.

The increased and prolonged maternal provisioning during embryonic development takes place in several animal groups through the placenta (Wood & Oakey 2006) or placenta-like structures. These are organs that allow a close association between mother

and offspring tissues, thus promoting an efficient physiological exchange of nutrients, gases and excretions (Mossman 1991; Wooding & Burton 2008). Placentae can be found in mammals and in other vertebrates such as sharks, fish and reptiles (Amoroso 1968; Renfree 1982; Lombardi & Wourms 1985a; Kwan et al. 2015). Placentation is commonly considered to be mainly a mammalian attribute, yet it evolved first in fish (Wourms & Lombardi 1992), and several other groups (reptiles, amphibians and plants) have also independently evolved placenta-like structures (Reznick et al. 2002; Haig & Westoby 1991; Blackburn 1999) which in some cases are also called placenta. Such is the case of the trophotaenial placenta of Goodeid fish, a product of modifications of maternal and embryonic cells and tissues that facilitate maternal-embryonic nutrient transfer (Lombardi & Wourms 1985a; Lombardi & Wourms 1985b), and promotes an increase of embryonic dry weight of up to 38,700 % through gestation (Lombardi & Wourms, 1979).

Although the primary function of all placentae is the mother-embryo nutrient and waste transfer (Faber et al. 1992), it is one of the most morphologically and diverse organs among the animals (Mossman 1991), including mammals (Mossman 1987; Faber et al. 1992; Wildman 2011), reptiles (Thompson & Speake 2006) and fish (Wourms 1981; Wourms et al. 1988), and it also exhibits great physiological diversity. Placentae constitute a functional link in the transition from lecithotrophy to matrotrophy, and provide the physiological scenario in which conflict can be expressed. Thus, Klisch and Mess (2007) proposed that the divergent interests between mother and offspring, followed by a rapid antagonistic coevolution, were the main causes of the evolution of the mammalian placenta, whereas Chuong and co-workers (2010) suggested that the mammalian placental proteins that mediate mother-embryo interactions may often be targets of evolutionary conflict. Furthermore, there is evidence that most imprinted genes -those that are expressed in a parent-of-origin manner; see below- are expressed in the placenta (Kaneko-Ishino et al. 2003), and some of them are essential for placental development and growth (Baker et al.

1993; Renfree et al. 2012). Among these genes, the ones that increase embryonic growth are usually paternally expressed whereas those that tend to restrict growth are maternally expressed (Renfree et al. 2012); therefore, some authors have attributed the rapid diversification of the placenta to the mother-offspring conflict and its consequent antagonistic coevolution (Reviewed in Pollux et al. 2014).

Re-tracing the path from oviparity to matrotrophic viviparity

Theories proposed to explain the evolution of viviparity, matrotrophy and placentation are diverse and often contradictory. However, it is unlikely that a single explanation holds for the complex, many-stage transition from oviparity to matrotrophic viviparity. More likely, it must have been shaped by a variety of selective pressures that interacted simultaneously or sequentially, and even such interactions or sequences of events may have differed in the distinct lineages that became matrotrophic -thus promoting lineage diversification-. Typically, reviews and theoretical papers have focussed on only one, or a few, of the evolutionary transitions that must have occurred between oviparity and matrotrophic viviparity. Here we present a possible pathway by which natural selection and genetic conflicts of interest may have driven the evolution of matrotrophy from an oviparous ancestral stage (Fig. 1).

Internal fertilisation. Following the idea that females are generally the limiting sex, as their reproductive output is normally set by the amount of eggs they can produce, whereas that of the males is determined by how many female eggs they have the capacity to fertilise (Trivers 1985), there is a premium for males to gain access to fertile females before other males do. This leads to protandry, by which males emerge/arrive at the breeding ground before females (Wiklund & Fagerström 1977). This form of male-male competition that favours males to be prepared to mate before their rivals would, in externally-fertilising species, promote a heightened readiness to ejaculate just as- or shortly before spawning.

In the extreme, any male attribute that can allow males to deliver sperm directly into the female reproductive tract before egg laying would be favoured by intrasexual selection, even in the absence of any female adaptation for egg retention. Accordingly, it has been proposed that internal fertilisation evolved because it reduces the intensity of sperm competition (Parker 1970). Alternatively, internal fertilisation could have evolved even in the absence of sperm competition, as it can reduce the risk of sperm being lost, and hence increase the probability that eggs are fertilised. This would be beneficial for both the males, as their sperm would not be wasted, and females, as it ensures the fertilisation of their ova (Parker 1984). If the driving force was reducing the intensity of sperm competition, then internal fertilisation might have evolved through intra-sexual conflict, as male competition would determine paternity (in the absence of cryptic female choice). However, the hypothesis that fertilisation was the driving force behind the evolution of internal fertilisation in several cases does not involve conflict, as all participants benefit from it. We note that internal fertilisation does not necessarily involve copulation, which it may often precede (see Parker 1984), and whose evolution may have been guided by sexual conflict (when coercive means such as claspers have evolved; Shibukawa et al. 2012) or not. Internal fertilisation is found in many fish and amphibian taxa where eggs are not retained (Townsend et al. 1981; Halliday 1994), such as in many oviparous urodele amphibians (Watanabe & Onitake 2002), or in several fish families (Burns et al. 1995; Pecio & Rafinski 1999), and in birds (Halliday 1994). Indeed, adaptations for internal fertilisation in oviparous fish can take the form of extreme head modifications that allow males to grasp females during sperm transfer, and which seem wholly unrelated to egg retention (e.g. priaprium fish; Shibukawa et al. 2012).

Egg retention, on the other hand, is considered a result of adaptations to counteract the effect of harsh environmental factors and pre-hatching predation risks (natural selection pressures; Andrews & Rose 1994). According to this view, increased offspring survival would have followed from their developing within the mother, hence in a

more secure and stable environment that also accelerated their development or that allowed females to enhanced their offspring's fitness by manipulating some of their phenotypic traits (reviewed by Shine 2014). This may sometimes have been the case, but we note that harsh environments are just as likely to promote the production of resistant eggs that can survive hard environmental conditions, even if their parents themselves cannot. This, for instance, is the case of annual fishes (Cyprinodontiformes; Murphy et al. 1999) a diverse group of tropical minnows that inhabit ephemeral water bodies, which they re-populate each wet season from draught-resistant eggs (Rodao et al. 2015). Hence rather than promoting egg retention, harsh environments may lead to the production and laying of even more independent eggs. Thus, as with internal fertilisation, egg retention may have evolved in response to different selective pressures, some of which may be related to sexual conflict. It may, in fact, be a consequence of internal fertilisation, if males can somehow promote embryonic development before eggs are laid, either by influencing their growth rate (see below), or by manipulating females into delaying oviposition (just as insect male ejaculates can induce oviposition; e.g. Yamane & Miyatake 2010; see Cordero 1995). No such mechanism has been described in vertebrates, yet sporadic accounts of developing fish embryos being laid by otherwise oviparous species (e.g. Hayakawa & Munehara 2001; Hayakawa & Munehara 2003) suggest that once internal fertilisation occurs, internal gestation can evolve, and since this transition would benefit the embryo's sire, male adaptations may evolve to promote female egg retention.

Staggered embryo provisioning. In internally-fertilising egg laying species insemination if often followed by a short-term egg retention during which the already yolked egg receives additional nutrients -which in monotremes has been likened to a brief period of matrotrophy; see below- and a hard protective shell. Thus long-term egg retention requires that a hard shell is not deposited (or that it is laid down and removed, but this seems unlikely), since it would impede gas exchange, and could injure the female if accidentally

broken. This is in essence the situation in lecithotrophy, where the mother makes most of the investment in nutrients during the production of the egg, and then allows it to develop inside her body. Mothers in lecithotrophic species face two constraints. 1) Their provisioning of resources to the clutch can be reduced if conditions deteriorate (a process akin to avian adaptive brood reduction), but it cannot be increased if conditions improve. 2) They are encumbered with the fully provisioned eggs through the gestation period. Those constraints place a premium on the production of eggs with little or no yolk, which then gradually become supplied through gestation. This allows females to carry the biomass of the full clutch for only a fraction of the gestation period -hence reducing the energetic cost of mobility and the period of greater vulnerability to predation-, and to adjust the rate at which she delivers resources to the embryos in response to changes in ecological conditions. We will refer to as matrotrophy the occurrence of female adaptations to control the flow of resources to the developing embryos. Full maternal control of this process may not be adaptive to the embryos, or to all of the embryos in a clutch, and this may lead to a departure from the honest signalling of embryonic needs (see Godfray 1995; Haig 1996) and to the evolution of means that allow the embryos to control the flow of resources from the mother, such as embryonic placentae. Thus, although at first glance, staggered provisioning might be beneficial for both the mother and the embryo, it may not be evolutionarily stable, being rather open to invasion by manipulative strategies from either the embryos or the father(s) of the clutch.

Although normally pooled under the term matrotrophy, we think that a distinction is necessary between 1) maternal control of the gradual delivery of resources to the embryos (matrotrophy *sensu stricto*), and the situation where there are adaptations in the embryos that permit the control of the maternal physiology resulting in staggered provisioning according to the embryos' interests. We propose that the term embryotrophy is used for the latter (and that ovotrophy is used for both lecithotrophy and for the process of embryo

nutrition through uterine fluid in the egg case, for which the term embryotrophy was used earlier by Castro et al. 2016).

Placental diversity, which can be extensive in some taxa, might be due to divergent adaptation to a variety ecological conditions calling for special requirements in the transport of nutrients. This, however, seems unlikely, since the environment experienced by, say, zebras and gazelles is essentially the same, whereas their placentae are widely different (diffuse and polycotyledonary respectively). Considerations such as these led Crespi & Semeniuk (2004; see also Klisch & Mess 2007) to propose that antagonistic parent-offspring coevolution might explain the differentiation of placental types within and across taxa (see Uribe & García Alarcón 2005; Mess & Carter 2007; Pollux et al. 2014). Here we have extended this rationale, and propose that in addition to parent-offspring conflict, the evolutionary conflict between sexes can help understanding the origin of viviparity in vertebrates, as well as the transitions between different forms of viviparity. Furthermore, we propose that tracing on the phylogeny of vertebrates the steps from oviparity to advanced forms of viviparity would reveal the conditions in which maternal, paternal or offspring control over provisioning has evolved and would allow us to predict in what groups mother-offspring conflict or sexual conflict could have evolved.

Mating systems and conflict

The occurrence and intensity of reproductive conflict between sexes varies across mating systems. These are traditionally classified as monogamous (breeding units formed by one female and one male), polygamy, divided into polygyny (one male mates with several females that it monopolises) and polyandry (one female mates with several males that she monopolises), polygynandry and promiscuity. In all systems it may be that the members of the breeding group remain together for several breeding seasons, or that they form new breeding alliances in different breeding events (Emlen & Oring 1977). Under strict (i.e.

genetic) monogamy, both parents obtain the same fitness from each offspring produced in common. This coincidence of reproductive interests discourages -although it does not preclude- the manipulation of the partner's breeding effort. Only if the parents mate for life their long-term reproductive interests are completely overlapped. By contrast, when the individuals pair with different partners in successive breeding events, their long-term interests differ, so that manipulation of the partner's current breeding effort can be beneficial even if it reduces the residual reproductive value of its mate. A similar situation occurs in polygynous mating systems if females move between breeding units in different breeding seasons. This may not be the case in polyandry, as defined above, since male manipulation of maternal provisioning may result in more resources being delivered to the offspring of competing males. Additionally, polyandrous females often extract more resources from the males, through receiving several nuptial gifts (Gwynne 1984; Zeh & Smith 1985; see Eberhard 1996; Reynolds 1996 for fitness consequences of gifts) or gaining access to various male territories (Davies & Hatchwell 1992). Both types of polygamous mating systems can be beneficial for males and females alike, as they may reduce the risk of genetic incompatibility (Zeh & Zeh 1996; Zeh & Zeh 1997) or inbreeding (Brooker et al. 1990; Stockley et al. 1993).

While a particular mating system may set the scenario in which sexual conflict over the provisioning of offspring can evolve, the specific way in which it is expressed depends on the reproductive mode. The existence of maternal or embryonic means to stagger the delivery of resources through pregnancy opens an opportunity for the evolution of mechanisms to bias the process; an opportunity that has not been evolutionarily missed.

Genomic imprinting

The kinship theory of genomic imprinting

Genomic imprinting is a widespread phenomenon, in which certain genes are expressed in a parent-of-origin manner, that is, the two parental alleles are expressed differentially (Tilghman 1999). This type of epigenetic gene regulation is found in animals (eutherians and marsupials, but apparently not in monotremes or birds; DeChiara et al. 1991; O'Neill et al. 2000; Killian et al. 2001; Constância et al. 2002) and in plants (Lin 1984; Grossniklaus & Vielle-Calzada 1998; Scott et al. 1998) and results in a monoallelic gene expression (in animals) or in a ploidy ratio of 2m:1p (maternal and paternal respectively) in some tissues (e.g. endosperm) in plants.

Trivers (1974) was the first to mention the possibility of a conflict between parents and offspring related to differing genetic interests, which could drive offspring to employ physiological weapons to manipulate maternal investment. During pregnancy, different sources of genetic conflict may rise: i) between genes expressed in the mother and in the foetus/placenta, or ii) between maternally-derived and paternally derived genes within the embryonic genome (Haig 1996). Whenever there are mother-offspring interactions, four sources of genes can be recognisable: a) genes expressed in the mother, b) genes expressed in the offspring, c) maternally inherited genes expressed in the offspring, and d) paternally inherited genes expressed in the offspring (see Crespi & Semeniuk 2004).

The evolution of the gene expression type (a) and (b) may be shaped by a process of antagonistic coevolution between mother and embryo. Under this scenario a genetic conflict may arise between maternal and foetal genes, where expression of the latter will be selected to increase the transport of maternal nutrients, and maternal genes will be selected to be expressed so that nutrient transport takes place at a level that is optimal for the mother (Haig 1993). Indeed, in mammals, hormones produced by the embryonic placenta may be interpreted as foetal attempts to manipulate maternal metabolism for the offspring's benefit (Haig 1996). Gene expression type (c) and (d), on the contrary, may reflect a conflict between parental alleles expressed in the offspring, where paternally derived alleles will be

selected to favour a more efficient nutrient acquisition and maternally derived alleles will be selected to favour an even distribution of maternal resourced among broods (Haig & Westoby 1989). This kind of conflict has been interpreted as the force that drove the evolution of genomic imprinting.

The Kinship Theory of genomic imprinting mainly proposes the parent-of-origin gene expression evolved as a consequence of a conflict between the interest of the paternally-inherited alleles (padumnal alleles) and maternally-inherited alleles (madumnal alleles) over maternal investment during offspring development (Moore & Haig 1991; Haig 2000a).

According to the Kinship theory, because the conflict is associated to maternal allocation of resources, it is predicted that the genes that increase nutrient demand from the mother will be paternally expressed and the genes that restrict embryo growth will be maternally expressed (Renfree et al. 2012; Haig 1996; Haig 2000a). This is expected to evolve when: 1) offspring are sired by more than one father, either among litters or in the same litter, 2) Provisioning of offspring during development is largely performed by the mothers, and 3) there are genes expressed on the offspring that can manipulate the amount of resources that the mother provides (Wilkins & Haig 2003).

Maternal resources for embryos are finite, therefore females must trade offspring number versus size (Marshall et al. 2008), and current vs. future reproduction (involving survival and growth; Stearns 1992). Producing large, healthy offspring may be beneficial for both parents, but females may be better off sometimes by having smaller offspring if excessive foetal demands lead to decreased female growth or survival, thus compromising future reproductive events (Haig 1996).

A classic example of genomic imprinting that is consistent with kinship theory is the expression pattern of the Insulin-like Growth Factor 2 (*igf2)* and its receptor (*igf2r*). IGF2 is a protein that promotes growth and cellular differentiation during development (Cohick & Clemmons 1993). It also regulates the placental supply of nutrients, and the genetic demand

for nutrients by the foetus (Constância et al. 2002; Fowden et al. 2006), and its over expression has been related to some overgrowth disorders in humans (Morison & Reeve 1998). On the other hand, *igf2r* encodes a membrane protein (cation independent mannose-6-phosphate receptor) that captures and transports the excess the IGF2 to the lysosomes for posterior degradation (Kornfeld & Mellman 1989), and thus it is essential for regulating normal foetal growth, circulating level of IGF2, and heart development (DeChiara et al. 1991; Lau et al. 1994). In therian mammals, the paternal allele of *igf2* is expressed and the maternal allele is silent (DeChiara et al. 1990) while *igf2r* is maternally active and paternally silent (Barlow et al. 1991). This accords to the proposal by Moore and Haig (1991) that if a mutation on a paternally expressed locus that can extract more nutrients from the mother evolves, then it will have a selective advantage over the other alleles because it will be able to extract more nutrients for its bearer. As a consequence, a maternally expressed allele that can reduce those demands would evolve as a counter-adaptation

Igf2 is imprinted in eutherians and marsupials, but not in monotremes or birds (O'Neill et al. 2000; J. Keith Killian et al. 2001; Nolan et al. 2001), and *igf2r* is imprinted in marsupials and most eutherians but not in birds, monotremes and primates (Killian et al. 2000; Clairmont & Czech 1989). The existence of a binding site for *igf2* in the IGF2R of marsupials and eutherians (Dahms et al. 1993; Yandell et al. 1999), but not in the IGF2R of birds, frogs and monotremes (Killian et al. 2000; Clairmont & Czech 1989), supports the idea of IGF2R evolution as a females' counter-adaptation to mitigate the effects of an excess of IGF2 (Wilkins & Haig 2003).

Conflict and genomic imprinting

If there is a conflict between parents and offspring who share half of their genomes, the conflict of interest between sexes is even stronger as a consequences of the lack of relatedness between mates (Dawkins 1989).

Haig (2000) proposed the Kinship Theory to explain why in biallelic organisms some alleles are expressed in a parent-of-origin manner. He established that the conflict between parental alleles is a result of the differing genetic interests between them (Haig 2014). *Igf2* monoallelic expression, for example, has been linked to the conflict between the paternal and the maternal genomes regarding the maternal allocation of resources during embryonic development. Nonetheless, this kind of conflict can also be extrapolated to sexual conflict, since *igf2* encodes a protein that controls the demands for nutrients in the embryo, and the nutrients delivery in the placenta (Constância et al. 2002), and the cost of a higher maternal investment imposed by the increment of the paternal protein are only faced by females. This makes the interest of both sexes diverge and clash, especially if fathers do not sire every litter of a female, leading an evolutionary arms race between sexes that takes place at a genomic level and reflects allele conflict, as Haig (2000) suggested. In an antagonistic coevolution between adaptations (in this case gene expression level) of the sexes, equilibrium could be reached if adaptations allow both sexes to maximise their own fitness. We could consider the conflict between parental alleles as a part of the conflict between males and females that is present in viviparous polyandrous systems, because it is difficult to establish if the alleles conflict directly with each other, or if the alleles' evolution is affected by the sexual conflict.

As mentioned elsewhere, it has been suggested that genomic imprinting is a characteristic exclusive of viviparous mammals that appeared before the marsupials and eutherian split, and that evolved differentially in both groups, resulting in a larger number of imprinted genes in eutherians compared to marsupials (Renfree et al. 2012; Fig. 2). Thus the evolution of genomic imprinting as a result of conflict between parental alleles, or between the sexes, over maternal allocation of resources has been extensively studied in mammals. However, viviparity and maternal provisioning of resources during pregnancy (the main enhancers of this type of conflict), have been also documented in many vertebrate

taxa, and thus genomic imprinting -and any other attribute of viviparity- can be expected to have evolved independently many times through the lineage of the vertebrates.

Internal gestation in vertebrates.

Internal gestation has independently evolved in separated taxonomic groups [Chondrichthyans (sharks and rays; 420 species grouped in 40 families), Sarcopretigiian fish (Coelacanth, one family), Actinopterygians (13 families and 510 species) amphibians (some salamanders, some frogs and 75% of caecilians), squamate reptiles (many lizards and snakes; reviewed in Blackburn 1999; Wourms 1981)], and both internal fertilization and viviparity first appeared in the vertebrate line in the extinct Placodermi (Long et al. 2008; Long et al. 2009), while all the known structures and adaptations for maternal-foetal nutrient transfer evolved first among fishes (Wourms 1981). Additionally, substantial matrotrophy/embryotrophy has originated in at least 24 occasions among viviparous vertebrates, mainly among fish (Blackburn et al. 1985; Blackburn 1992). Thus, we shall devote a few words to the evolution of viviparity in amphibians and reptiles, before delving into the evolution of viviparity in fish (see supplementary material).

An extended mode of amphibian reproduction involves the occurrence of a larval stage, which in several cases can undergo metamorphosis before hatching. Embryo provisioning may take the form of nutrients (eggs) passed to the larvae (Buckley 2012). This allows the mother to control a staggered delivery of resources to the developing young, and there is little opportunity for the latter (or the father) to control the female investment in their own benefit. The embryos of viviparous salamanders and caecilians sometimes have specialised teeth that scrap the maternal tissues (Buckley 2012). This form of embryotrophy (known as matrophagy) may preclude the evolution of an embryonic placenta and in some salamanders is preceded by an oophagous phase. Oophagy may allow the female to determine the maximum investment she is willing to make in a particular reproductive effort, while the embryos regulate the tempo at which the resources are consumed. Embryophagy,

on the other hand, limits the control that can be exerted by the female on the rate at which resources are passed from the mother to the offspring, since embryos can ingest their own viable siblings to which females have allocated nutrients during gestation. Although this form of amphibian embryotrophy is exclusive of *Salamandra salamandra* (Buckley et al. 2007; Buckley 2012) and little is known about its mating system, we predict a stronger selective pressure for the evolution of embryophagy in polygamous mating systems with multiple paternity, where sibling ingestion is more beneficial than costly to embryos, and is particularly costly to females, but not to males.

Histophagy (see Box 1), that has evolved in some salamandrids (Blackburn 2015) and bufonids (Xavier 1973; Wake 1980), and histotrophy (see Box 1) that occurs in some members of *Hemiphractidae* (Savage 2002; Roberts et al. 2016) should allow females to control the nutrient delivery; however, in some amphibians such as caecilians and salamandrids, embryos can stimulate maternal secretions by abrading the uterine lining with their prenatal teeth (Wake 1977; Guex & Chen 1986), and in some anurans (i.e. bufonids), the oviductal secretions are controlled by embryogenic hormones (Xavier et al. 1970), whose production may conceivably be influenced by the father.

Viviparity has appeared 115 times among the reptiles (Blackburn 2015). The squamate placenta is characterized by the apposition of the chorioallantoid and specialised derivatives of the yolk sac (embryonic component) to the uterine lining (maternal component; Stewart & Blackburn 1988), and is responsible for a significant transfer of nutrients in highly matrotrophic? squamates (Blackburn 2015). Most viviparous squamates are relatively lecithotrophic and transfer small quantities of nutrients through the placenta via histrotrophy (Blackburn 1994; Stewart & Thompson 2000; Thompson & Speake 2006); nevertheless, substantial matrotrophy has evolve in 6 clades of scincid lizards such as those in the genera *Mabuya* and *Chalcides* (Blackburn 1992; Stewart & Blackburn 2014). In particular, lizards of the genera *Mabuya* have long gestation periods (8-12 months), during which the mothers

provide large amounts of nutrients to the embryos via placental means that allow the embryo to have a dry mass increase of 38 400% (Blackburn et al. 1984; Blackburn 1992).

All squamate placentae have a maternal component (uterine epithelium) and an embryonic component (chrorioallantois or specialised derivatives of the yolk sac), and in the case of species of the genera *Pseudemoia*, *Mabuya* and *Eumecia*, they also have the placentome; a structural development of a specialised part of the chorioallantoic placenta, whose maternal component appears to be secretory (Thompson et al. 2004; Adams et al. 2005), while the embryonic one is absorptive (Blackburn 2000).

Social monogamy is uncommon among reptiles (Harrison 2013) and multiple paternity, which can involve as many as 50% of all litters, has been documented in all lizard and snake species investigated so far (Uller & Olsson 2008). Thus, if maternal-foetal communication in squamate reptiles is regulated chemically, and offspring signal their necessities via hormones synthesised in / released by the placentome, then both the embryo and (or) the father may influence maternal resource allocation.

Fish are characterized by a continuous progression of viviparous species, from cases where mothers do little more than merely protecting the ova, to true viviparous species, where the nutritional, respiratory and excretory demands of the embryos are satisfied by the mother (Amoroso 1960; Wourms & Lombardi 1992) through structures such as placental analogues or ovarian nipples (Turner 1940; Lombardi & Wourms 1979; Blackburn 2015), or via oophagy, embryophagy, histotrophy and histophagy. Substantial matrotrophy has evolved in at least nine of the 12 clades of viviparous teleosts, and in four of the eight clades of viviparous elasmobranchs (Blackburn 2015). Embryos can absorb or ingest nutrients deposited in the ovarian lumen or the ovarian follicle across permeable surfaces.

Placentotrophy is common among viviparous teleosts (see supplementary material) and can be supplemented by histotrophy or histophagy. However, it can also be accompanied by oophagy and in some cases by embryophagy (Wourms 1981; Greven &

Grossherr 1992; Blackburn 2015). Placentotrophy is also present in several Condrichthyes (mainly in Elmasmobranchii and in few Holocephalii; Hamlett et al. 1996; López et al. 2006; Carrier et al. 2012; Roberts et al. 2016) that show elaborated maternal and foetal specialisations for nutrient provision and absorption (Hamlett et al. 1985; Hamlett et al. 1993)

Although oophagy is widely distributed among viviparous fishes (Blackburn 2015) and the mother can control the maximum quantity of nutrients she provides, allocation mediated by placentae, histrotrophy and histophagy, that may be influenced again by the embryos or the father, have evolved in most viviparous teleosts and carcharhiniformes (see appendix). Additionally, the evolutionary foundation of genomic imprinting has been demonstrated in oviparous fish (Xie et al. 2009), and there is evidence that insulin-like growth factor 2 is under positive selection in fish, which coincides with the evolution of placentation in this group (O'Neill et al. 2007). Moreover, spontaneous abortion rate produced by crosses between populations with a different level of polyandry in poecilid fish has been related to the postzygotic reproductive isolation between populations as a product of parent-offspring conflict (Schrader & Travis 2008).

The above suggests that the same type of conflict and antagonistic coevolution that has been documented in mammals may be present in many other organisms under the same strong conflict related to maternal allocation of resources and with the same mating system. Therefore, we consider that more research is needed in viviparous groups other than mammals in relation to genomic imprinting on genes in charge of embryo-maternal communication and nutrient transfer. According to the genomic imprinting conflict theory, the paternally expressed alleles should promote growth while maternal alleles should restrain it. Perhaps *igf2* is the most studied and best described example of genomic imprinting and conflict in mammals, nevertheless, there are many other genes that enhance and restrict growth during development in mammals (Table 1). Additionally, since genomic

imprinting has not evolved in the same way even among organisms of the same group (e.g. *Igf2r, U2afbp-rs* and *Impact* are imprinted in mouse, but not in humans; Kalscheuer et al. 1993; Pearsall et al. 1996; Okamura et al. 2000), it is likely even if genomic imprinting of important genes, such as *igf2*, does not occur in all the matrotrophic groups, this does not necessarily mean that imprinting as a result of sexual conflict over maternal allocation of resources has not evolved in some other growth-related genes, because almost none of them have so far been tested in other viviparous and more matrotrophic organisms than mammals.

The bewildering diversity of adaptations surrounding viviparity in vertebrates may obscure any underlying evolutionary pattern. Still, as mentioned earlier, we propose that certain sequential transitions are more likely to have occurred than others (e. g. ovoviviparity – matrotrophic viviparity), and that conflict of interests between the participants (mother, father, embryos) would have promoted diversity, as the outcomes would vary from one instance to other. Both sets of predictions require comparative analyses. We are, however, still ignorant of the ways in which internal gestation works in most of the taxa where it occurs, and appropriate phylogenies at the family or sub-family level are often lacking. Nevertheless, we conducted a preliminary exercise of tracing several features of viviparity on the phylogenies of vertebrates (see Supplementary material) to see whether the sequence of events that led to the most complex forms of staggered embryo provisioning, and whether mother or offspring/father lead in the resolution of the conflict, can be inferred, and if so, whether such patterns accord with our proposal (as stated above). We also evaluate whether diversity of processes/taxa is higher in lineages where internal gestation appears.

Conclusions and future directions

Reproduction is costly, and selection has rewarded those organisms that can manipulate their partner to invest more than their fair share in raising their common offspring. The

evolution from oviparity to matrotrophic viviparity and the apparition of a great diversity of placentae has been gradual and was probably shaped by several forces of natural and sexual selection acting simultaneously. It is likely that the evolution of egg retention and the subsequent matrotrophic viviparity were favoured predominantly by natural selection pressures for increasing offspring development and survival and for decreasing the mother predation risk and the possibility of the loss of the brood. Yet the evolution of the enormous variety of placentae (and maybe of species among viviparous vertebrates) was probably due to the substantial conflict of interests between mother and offspring, and between mother and father -expressed in the genome of their offspring- regarding the optimal maternal allocation of resources during offspring development.

Genomic imprinting of genes responsible for embryonic growth has rarely been tested in viviparous polyandrous animals other than mammals. Although *igf2* is expressed biallelically in placental poeciliid fish, we found evidence suggestive of epigemetic regulation of *igf2* expression and genomic imprinting of *igf2* in this family (Saldivar Lemus et al. 2017). Since the opportunity for the evolution of similar mechanisms exists in amphibians and reptiles, we predict that genomic mechanisms of manipulating maternal allocation will also be found among them.

References

Adams, S.M. et al., 2005. Cyto- epitheliochorial placenta of the viviparous lizard Pseudemoia entrecasteauxii: a new placental morphotype. *Journal of Morphology*, 264(3), pp.264–276.

Alexander Pyron, R. & Wiens, J.J., 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, 61(2), pp.543–583.

Amoroso, E.C., 1968. The Evolution of Viviparity. *Proceedings of the Royal Society of Medicine*, 61(11 Pt 2), pp.1188–1200.

Amoroso, E.C., 1960. Viviparity in fishes. *Symp. Zool. Soc. London (Vol.*, 1, pp.153–181.

Andrews, R.M. & Rose, B.R., 1994. Evolution of viviparity: constraints on egg retention. *Physiological Zoology*, 67(4), pp.1006–1024.

Arnqvist, G. & Rowe, L., 2002. Antagonistic coevolution between the sexes in a group of insects. *Nature*, 415, pp.787–789.

Aschliman, N.C. et al., 2012. Body plan convergence in the evolution of skates and rays (Chondrichthyes: Batoidea). *Molecular Phylogenetics and Evolution*, 63(1), pp.28–42.

Baker, J. et al., 1993. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell*, 75(1), pp.73–82.

Barlow, D.P. et al., 1991. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature*, 349, pp.84–87.

Blacher, P., Huggins, T.J. & Bourke, A.F.G., 2017. Evolution of ageing, costs of reproduction and the fecundity – longevity trade-off in eusocial insects. *Proceedings of the Royal Society B: Biological Sciences*, 284(1858), p.20170380.

Blackburn, D.G., 2000. Classification of the Reproductive Patterns of Amniotes. *Herpetological Monographs*, 14, pp.371–377.

Blackburn, D.G., 1992. Convergent Evolution of Viviparity, Matrotrophy, and Specializations for Fetal Nutrition in Reptiles and Other Vertebrates. *American Zoologist*, 32(2), pp.313–321.

Blackburn, D.G., 2015. Evolution of vertebrate viviparity and specializations for fetal nutrition: A quantitative and qualitative analysis. *Journal of Morphology*, 276(8), pp.961–990.

Blackburn, D.G., 2000. Reptilian viviparity: Past research, future directions, and appropriate models. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 127(4), pp.391–409.

Blackburn, D.G., 1994. Standardized Criteria for the Recognition of Embryonic Nutritional Patterns in Squamate Reptiles. *Copeia*, 1994(4), pp.925–935.

Blackburn, D.G., 1999. Viviparity and oviparity: Evolution and reproductive strategies. *Encyclopedia of Reproduction*, 4, pp.994–1003.

Blackburn, D.G. & Evans, H.E., 1986. Why are there no viviparous birds? *The American Naturalist*, 128(2), pp.165–190.

Blackburn, D.G., Evans, H.E. & Vitt, L.J., 1985. The evolution of fetal nutrititional adaptations. *Fortschr. Zool.*, 30(JANUARY 1985), pp.437–439.

Blackburn, D.G., Vitt, L.J. & Beuchat, C. a, 1984. Eutherian-like reproductive specializations

in a viviparous reptile. *Proceedings of the National Academy of Sciences of the United States of America*, 81(15), pp.4860–4863.

Brooker, M. et al., 1990. Promiscuity: an inbreeding avoidance mechanism in a socially monogamous species? *Behavioral Ecology and Sociobiology*, 26(3), pp.191–199.

Buckley, D., 2012. Evolution of Viviparity in Salamanders (Amphibia , Caudata). *Enciclopedia of Life Sciences*, (April 2012), pp.1–5.

Buckley, D. et al., 2007. Heterochrony, cannibalism, and the evolution of viviparity in Salamandra. *Evolution & Development*, 9(1), pp.105–115.

Buckling, A. & Rainey, P.B.., 2002. Antagonistic coevolution between a bacterium and a bacteriophage. *Proceedings of the Royal Society B: Biological Sciences*, 269(1494), pp.931–936.

Burley, N., 1981. Sex ratio manipulation and selection for attractiveness. *Science*, 211(4483), pp.721–722.

Burns, J.R. et al., 1995. Internal fertilization, testis and sperm morphology in glandulocaudinae fishes (Teleostei: Characidae: Glandulocaudinae). *Journal of Morphology*, 224(2), pp.131–145.

Carrier, J.C., Musick, J.A. & Heithaus, M.R., 2012. *Sharks and Their Relatives II: Biodiversity, adaptive physiology and conservation*, CRC press.

Castro, J.I., Sato, K. & Bodine, A.B., 2016. A novel mode of embryonic nutrition in the tiger shark, *Galeocerdo cuvier*. *Marine Biology Research*, 12(2), pp.200–205.

Chapman, T. et al., 2003. Sexual conflict. *Trends in Ecology & Evolution*, 18(1), pp.41–48.

Chuong, E.B., Tong, W. & Hoekstra, H.E., 2010. Maternal-Fetal Conflict: Rapidly Evolving Proteins in the Rodent Placenta. *Molecular Biology and Evolution*, 27(6), pp.1221–1225. Clairmont, K.B. & Czech, M.P., 1989. Chicken and Xenopus Mannose 6-Phosphate Receptors Fail to Bind Insulin-like Growth Factor II. *Journal of Biological Chemistry*, 264(28), pp.16390–16392.

Cohick, W.S. & Clemmons, D.R., 1993. The Insulin-Like Growth Factors. *Annual Review of Physiology*, 55(1), pp.131–153.

Constância, M. et al., 2002. Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature*, 417(6892), pp.945–948.

Cordero, C., 1995. Ejaculate substances that affect female insect reproductive physiology and behavior : Honest or arbitrary traits? *Journal of Theoretical Biology*, 174(4), pp.453–461.

Crespi, B. & Semeniuk, C., 2004. Parent-offspring conflict in the evolution of vertebrate reproductive mode. *Am Nat*, 163(5), pp.635–53.

Davies, N.B. & Hatchwell, B.J., 1992. The Value of Male Parental Care and its Influence on Reproductive Allocation by Male and Female Dunnocks. *Journal of Animal Ecology*, 61(2), pp.259–272.

Dawkins, R., 1989. *The selfish gene* 6th Editio., New York: Oxford University Press.

DeChiara, T.M., Efstratiadis, A. & Robertson, E.J., 1990. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature*, 345, pp.78–80.

DeChiara, T.M., Robertson, E.J. & Efstratiadis, A., 1991. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell*, 64(4), pp.849–859.

Duvillié, B. et al., 1998. Imprinting at the mouse Ins2 locus: evidence for cis- and trans-allelic interactions. *Genomics*, 47(1), pp.52–7.

Duvillié, B. et al., 1997. Phenotypic alterations in insulin-deficient mutant mice. *Proceedings of the National Academy of Sciences of the United States of America*, 94(May), pp.5137–5140.

Eberhard, W.G., 1996. *Female Control: Sexual Selection by Cryptic Female Choice*, USA: Princeton University Press.

Emlen, S. & Oring, L., 1977. Ecology, sexual selection, and the evolution of mating systems. *Science*, 197(4300), pp.215–223.

Faber, J.J., Thornburg, K.L. & Binder, N.D., 1992. Physiology of Placental Transfer in Mammals. *American Zoologist*, 32(2), pp.343–354.

Fowden, a. L. et al., 2006. Imprinted genes, placental development and fetal growth. *Hormone Research*, 65(suppl 3), pp.50–58.

Frank, D. et al., 2002. Placental overgrowth in mice lacking the imprinted gene Ipl. *Proceedings of the National Academy of Sciences of the United States of America*, 99(11), pp.7490–7495.

Ghalambor, C.K., Reznick, D.N. & Walker, J. a, 2004. Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (Poecilia reticulata). *The American naturalist*, 164(1), pp.38–50.

Giddings, S.J. et al., 1994. Allele specific inactivation of insulin 1 and 2, in the mouse yolk sac, indicates imprinting. *Nature genetics*, 6(3), pp.310–313.

Gil, D. et al., 1999. Male Attractiveness and Differential Testosterone Investment in Zebra Finch Eggs. *Science*, 286(5437), pp.126–128.

Godfray, H.C.T., 1995. Signaling of Need between Parents and Young: Parent-Offspring Conflict and Sibling Rivalry. *American Naturalist*, 146(1), pp.1–24.

Greven, H. & Grossherr, M., 1992. Adelphophagy and oophagy in Ameca splendens Miller and Fitzsimons, 1971. *Zeitschrift fuer Fischkunde*, 12, pp.193–197.

Gross, M.T. & Sargent, R.C., 1985. The Evolution of Male and Female Parental Care in Fishes. *American Zoologist*, 25(3), pp.807–822.

Grossniklaus, U. & Vielle-Calzada, J.P., 1998. Response: Parental conflict and infanticide during embryogenesis. *Trends in Plant Science*, 3(9), p.328.

Guex, G.D. & Chen, P.S., 1986. Epitheliophagy: Intrauterine cell nourishment in the viviparous alpine salamander, Salamandra atra (Laur.). *Experientia*, 42(11–12), pp.1205–1218.

Gwynne, D.T., 1984. Courtship feeding increases female reproductive success in bushcrickets. *Nature*, 307, pp.361–363.

Haig, D., 2014. Coadaptation and conflict, misconception and muddle, in the evolution of genomic imprinting. *Heredity*, 113(2), pp.96–103.

Haig, D., 1993. Genetic conflicts in human pregnancy. *Quarterly Review of Biology*, 68(4), pp.495–532.

Haig, D., 1996. Placental hormones, genomic imprinting, and maternal-fetal communication. *Journal of Evolutionary Biology*, 9(3), pp.357–380.

Haig, D., 2000. The Kinship Theory of Genomic Imprinting. *Annual Review of Ecology and*

Systematics, (34), pp.9–32.

Haig, D. & Westoby, M., 1991. Genomic imprinting in endosperm - its effect on seed development in crosses between species, and between different ploidies of the same species, and its implications for the evolution of apomixis. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 333(1266), pp.1–13.

Haig, D. & Westoby, M., 1989. Parent-Specific Gene Expression and the Triploid Endosperm. *The American Naturalist*, 134(1), p.147.

Halliday, T., 1994. *Animal behavior*, Sidney: University of Oklahoma Press.

Hamlett, W.C. et al., 1996. Ultrastructure of uterine trophonemata, accommodation for uterolactation, and gas exchange in the southern stingray, Dasyatis americana. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 74(8), pp.1417–1430.

Hamlett, W.C. et al., 1993. Uterogestation and placentation in elasmobranchs. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 266(55), pp.347–367.

Hamlett, W.C., Wourms, J.P. & Hudson, J.S., 1985. Ultrastructure of the full-term shark yolk sac placenta. I. Morphology and cellular transport at the fetal attachment site. *Journal of Ultrasructure Research*, 91(3), pp.192–206.

Harrison, A., 2013. Size-assortative pairing and social monogamy in a neotropical lizard, Anolis limifrons (Squamata: Polychrotidae). *Breviora Museum of Comparative Zoology*, 534(534), pp.1–9.

Hayakawa, Y. & Munehara, H., 2003. Comparison of ovarian functions for keeping embryos by measurement of dissolved oxygen concentrations in ovaries of copulatory and non-copulatory oviparous fishes and viviparous fishes. *Journal of Experimental Marine Biology and Ecology*, 295(2), pp.245–255.

Hayakawa, Y. & Munehara, H., 2001. Facultatively internal fertilization and anomalous embryonic development of a non-copulatory sculpin Hemilepidotus gilberti Jordan and Starks (Scorpaeniformes: Cottidae). *Journal of Experimental Marine Biology and Ecology*, 256(1), pp.51–58.

Kalscheuer, V.M. et al., 1993. The insulin-like growth favtor type-2 receptor gene is imprinted in the mouse but not in humans. *Nature genetics*, 5, pp.74–78.

Kaneko-Ishino, T., Kohda, T. & Ishino, F., 2003. The Regulation and Biological Significance of Genomic Imprinting in Mammals. *Journal of Biochemistry*, 133(6), pp.699–711.

Killian, J.K. et al., 2000. M6P/IGF2R imprinting evolution in mammals. *Molecular cell*, 5(4), pp.707–716.

Killian, J.K. et al., 2001. Monotreme IGF2 expression and ancestral origin of genomic imprinting. *Journal of Experimental Zoology*, 291(2), pp.205–212.

Kleinteich, T. et al., 2012. Is solid always best ? Cranial performance in solid and fenestrated caecilian skulls. *Journal of Experimental Biology*, 215, pp.833–844.

Klisch, K. & Mess, A., 2007. Evolutionary Differentiation of Cetartiodactyl Placentae in the Light of the Viviparity-Driven Conflict Hypothesis. *Placenta*, 28, pp.353–360.

Kornfeld, S. & Mellman, I., 1989. The biogenesis of lysosomes. *Annu. Rev. Cell Biol.*, 5, pp.483–525.

Kwan, L. et al., 2015. An examination of the variation in maternal placentae across the genus Poeciliopsis (Poeciliidae). *Journal of Morphology*, 276(6), pp.707–720.

Lau, M.M.H. et al., 1994. Loss of the imprinted IGF2/cation-independent mannose 6-phosphate receptor results in fetal overgrowth and perinatal lethality. *Genes and Development*, 8(24), pp.2953–2963.

Lefebvre, L. et al., 1998. Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest. *Nature genetics*, 20(2), pp.163–169.

Li, L. et al., 1999. Regulation of maternal behavior and offspring growth by paternally expressed Peg3. *Science (New York, N.Y.)*, 284(April), pp.330–333.

Li, Y. & Behringer, R.R., 1998. Esx1 is an X-chromosome-imprinted regulator of placental development and fetal growth. *Nature genetics*, 20(3), pp.309–311.

Lin, B., 1984. Ploidy barrier to endosperm development i n maize. *Genetics Society of America*, 107, pp.103–115.

Lombardi, J. & Wourms, J.P., 1979. Structure, function, and evolution of trophotaeniae, placental analogues of viviparous fishes. *American Zoologist*, 19(3), p.976.

Lombardi, J. & Wourms, J.P., 1985a. The trophotaenial placenta of a viviparous goodeid fish. I: Ultrastructure of the Internal Ovarian Epithelium, the Maternal Component. *Journal of molecular biology*, 184, pp.277–292.

Lombardi, J. & Wourms, J.P., 1985b. The trophotaenial placenta of viviparous goodeid fish. II. Ultrastructure of the trophotaenia, the embryonic component. *Journal of Morphology*, 184(3), pp.293–309.

Long, J.A. et al., 2008. Live birth in the Devonian period. *Nature*, 453(7195), pp.650–652.

Long, J.A., Trinajstic, K. & Johanson, Z., 2009. Devonian arthrodire embryos and the origin of internal fertilization in vertebrates. *Nature*, 457(7233), pp.1124–1127.

López, J.A. et al., 2006. Phylogeny of sharks of the family Triakidae (Carcharhiniformes) and its implications for the evolution of carcharhiniform placental viviparity. *Molecular Phylogenetics and Evolution*, 40(1), pp.50–60.

Ludwig, T. et al., 1996. Mouse Mutants Lacking the Type 2 IGF Receptor (IGF2R) Are Rescued from Perinatal Lethality …. *Developmental Biology*, 535(182), pp.517–535.

Macías Garcia, C. & Ramirez, E., 2005. Evidence that sensory traps can evolve into honest signals. *Nature*, 434(7032), pp.501–505.

Magurrant, A.E. & Seghers, H., 2008. A cost of sexual harassment in the guppy , Poecilia reticulata. , 258(1351), pp.89–92.

Marshall, D.J., Bonduriansky, R. & Bussière, L.F., 2008. Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. *Ecology*, 89(9), pp.2506–2517.

McNamara, J.M. et al., 2003. Should young ever be better off with one parent than with two? *Behavioral Ecology*, 14(3), pp.301–310.

Mess, A. & Carter, A.M., 2007. Evolution of the placenta during the early radiation of placental mammals. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 148(4), pp.769–779.

Møller, P.R. et al., 2016. A new classification of viviparous brotulas (Bythitidae) - with family status for Dinematichthyidae - based on molecular, morphological and fossil data. *Molecular Phylogenetics and Evolution*, 100, pp.391–408.

Moore, T. & Haig, D., 1991. Genomic imprinting in mammalian development: a parental tug-of-war. *Trends in Genetics*, 7(2), pp.45–49.

Morison, I.M. & Reeve, A.E., 1998. Insulin-like growth factor 2 and overgrowth: Molecular biology and clinical implications. *Molecular Medicine Today*, 4(3), pp.110–115.

Mossman, H.W., 1991. Comparative Morphogenesis of the Fetal Membranes Accesso and Uterine Structures. *Placenta*, 12(1), pp.1–5.

Mossman, H.W., 1987. *Vertebrate fetal membranes: comparative ontogeny and morphology; evolution; phylogenetic significance; basic functions; research opportunities.*, Rutgers University Press.

Murphy, W.J., Thomerson, J.E. & Collier, G.E., 1999. Phylogeny of the Neotropical killifish family Rivulidae (Cyprinodontiformes, Aplocheiloidei) inferred from mitochondrial DNA sequences. *Molecular phylogenetics and evolution*, 13(2), pp.289–301.

Near, T.J. et al., 2012. Resolution of ray-finned fish phylogeny and timing of diversification. *Proceedings of the National Academy of Sciences*, 109(34), pp.13698–13703.

Nolan, C.M. et al., 2001. Imprint status of M6P/IGF2R and IGF2 in chickens. *Development Genes and Evolution*, 211(4), pp.179–183.

O'Neill, M.J. et al., 2000. Allelic expression of IGF2 in marsupials and birds. *Development genes and evolution*, 210, pp.18–20.

O'Neill, M.J. et al., 2007. Ancient and continuing Darwinian selection on insulin-like growth factor II in placental fishes. *Proceedings of the National Academy of Sciences of the United States of America*, 104(30), pp.12404–12409.

Okamura, K. et al., 2000. Comparative genome analysis of the mouse imprinted gene impact and its nonimprinted human homolog IMPACT: toward the structural basis for species-specific imprinting. *Genome Res*, 10(1220568479), p.1878–89.

Osorno, J.L. & Székely, T., 2004. Sexual conflict and parental care in magnificent frigatebirds: Full compensation by deserted females. *Animal Behaviour*, 68(2), pp.337–342.

Parenti, L.R., 2005. The phylogeny of Atherinomorphs: evolution of a novel fish reproductive system. In *Viviparous Fishes. New Life Publications, Homestead, …*. Homestead: New Life Publication, pp. 13–30.

Parker, A.G.A., 1974. Courtship Persistence and Female-Guarding as Male Time Investment Strategies. *Behaviour*, 48(1), pp.157–184.

Parker, G.A., 1970. Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, 45(4), pp.525–567.

Parker, G.A., 1984. Sperm Competition and the Evolution of Animal Mating Strategies. In *Sperm Competition and the Evolution of Animal Mating Systems*. pp. 1–60.

Patzner, R.A., 2008. Reprodictive strategies of fish. In M. Joao Rocha, A. Arukwe, & B. G. Kapoor, eds. *Fish reproduction. 311-250. (eds). Science Publishers, USA.* Science Publisher, pp. 211–250.

Pearsall, R.S. et al., 1996. Absence of imprinting in U2AFBPL, a human homologue of the imprinted mouse gene U2afbp-rs. *Biochem Biophys Res Commun*, 222(196212931), p.171–7.

Pecio, A. & Rafinski, J., 1999. Spermiogenesis in Mimagoniates barberi (Teleostei: Ostariophysi: Characidae), an oviparous, internally fertilizing fish. *Acta Zoologica*, 80(1), pp.35–45.

Pollux, B.J.A. et al., 2009. Evolution of Placentas in the Fish Family Poeciliidae: An Empirical

Study of Macroevolution. *Annual Review of Ecology, Evolution, and Systematics*, 40(1), pp.271–289.

Pollux, B.J.A. et al., 2014. The evolution of the placenta drives a shift in sexual selection in livebearing fish. *Nature*, 513, pp.233–236.

Pyron, R.A., Burbrink, F.T. & Wiens, J.J., 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology*, 13(1), p.93.

Renfree, M.B., 1982. Implantation and placentation. In C. R. Austin & R. V. Short, eds. *Reproduction in mammals pp. 26-69, 2*. Cambridge University Press, pp. 26–69.

Renfree, M.B. et al., 2012. The origin and evolution of genomic imprinting and viviparity in mammals The origin and evolution of genomic imprinting and viviparity in mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, p.20120151.

Reynolds, J.D.J., 1996. Animal breeding systems. *Trends in Ecology & Evolution*, 11(2), pp.68–72.

Reznick, D.N., Mateos, M. & Springer, M.S., 2002. Independent Origins and Rapid Fish Genus Poeciliopsis. *Science*, 298, pp.1018–1020.

Roberts, R.M., Green, J.A. & Schulz, L.C., 2016. The evolution of the placenta. *Reproduction*, 152(5), pp.R179–R189.

Rodao, M. et al., 2015. Sperm and Egg Envelope Ultrastructure and Some Considerations on Its Evolutionary Meaning. In *Annual Fishes: Life History Strategy, Diversity, and Evolution*. Boca Raton: CRC Press, pp. 47–62.

Saldivar Lemus, Y. et al., 2017. Asymmetric paternal effect on offspring size linked to parent-of-origin expression of an insulin-like growth factor. *Ecology and Evolution*, (April), pp.1–48.

Savage, J.M., 2002. *The Amphibians and Reptiles of Costa Rica: A Herpetofauna Between Two Continents, Between Two Seas*, Chicago: University of Chicago Press.

Schrader, M. & Travis, J., 2008. Testing the viviparity-driven-conflict hypothesis: parent-offspring conflict and the evolution of reproductive isolation in a poeciliid fish. *The American naturalist*, 172(6), pp.806–817.

Scott, R.J. et al., 1998. Parent-of-origin effects on seed development in Arabidopsis thaliana. *Development*, 125(17), pp.3329–3341.

Shibukawa, K., Tran, D.D. & Tran, L.X., 2012. Phallostethus cuulong, a new species of priapiumfish (Actinopterygii: Atheriniformes: Phallostethidae) from the Vietnamese Mekong. *Zootaxa*, 51(3363), pp.45–51.

Shine, R., 2014. Evolution of an Evolutionary Hypothesis: A History of Changing Ideas about the Adaptive Significance of Viviparity in Reptiles. *Journal of Herpetology*, 48(2), pp.147–161.

Smuts, B.B. & Smuts, R.W., 1993. Male Aggression and Sexual Coercion of Females in Nonhuman Primates and Other Mammals: Evidence and Theoretical Implications. *Advances in the Study of Behavior*, 22, pp.1–63.

Stearns, S.C., 1989. Trade-Offs in Life-History Evolution. *Functional Ecology*, 3(3), pp.259–268.

Stewart, J.R. & Blackb, 2014. Viviparity and placentation in lizards. In *Reproductive biology*

and phylogeny of lizards and tuatara. Florida: CRC Press, pp. 448–563.

Stewart, J.R. & Blackburn, D.G., 1988. Reptilian Placentation : Structural Diversity and Terminology. *Copeia*, 1988(4), pp.839–852.

Stewart, J.R. & Thompson, M.B., 2000. Evolution of placentation among squamate reptiles: recent research and future directions. *Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology*, 127(4), pp.411–431.

Stockley, P. et al., 1993. Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. *Proc. R. Soc. Lond. B*, 254, pp.173–179.

Tarwater, C.E. & Arcese, P., 2017. Young females pay higher costs of reproduction in a short-lived bird. *Behavioral Ecology and Sociobiology*, 71(5).

Thompson, M.B. et al., 2004. Placental function in lizards. *International Congress Series*, 1275, pp.218–225.

Thompson, M.B. & Speake, B.K., 2006. A review of the evolution of viviparity in lizards: structure, function and physiology of the placenta. *Journal of comparative physiology. B, Biochemical, systemic, and environmental physiology*, 176(3), pp.179–89.

Tilghman, S.M., 1999. The sins of the fathers and mothers: Genomic imprinting in mammalian development. *Cell*, 96(2), pp.185–193.

Townsend, D.S. et al., 1981. Internal fertilization in an oviparous frog. *Science*, 212(4493), pp.469–471.

Trexler, J.C., 1997. Resource availability and plasticity in offspring provisioning: Embryo nourishment in sailfin mollies. *Ecology*, 78(5), pp.1370–1381.

Trexler, J.C. & DeAngelis, D.L., 2003. Resource allocation in offspring provisioning: An evaluation of the conditions favoring the evolution of matrotrophy. *American Naturalist*, 162(5), pp.574–585.

Trivers, R.L., 1974. Parent-Offspring Conflict. *American Zoologist*, 14(1), pp.249–264.

Trivers, R.L., 1972. Parental Investment and Sexual Selection. In *Selection & the Descent of Man, , New York*. New York: Aldine de Gruyter, pp. 134–179.

Trivers, R.L., 1985. *Social Evolution*, Michigan: Cummings Pub. Co.

Turner, C.L., 1940. Pseudoamnion, pseudochorion, and follicular pseudoplacenta in poeciliid fishes. *Journal of Morphology*, 67(1), pp.59–89.

Tycko, B. & Morison, I.M., 2002. Physiological functions of imprinted genes. *Journal of Cellular Physiology*, 192(3), pp.245–258.

Uller, T. & Olsson, M., 2008. Multiple paternity in reptiles: Patterns and processes. *Molecular Ecology*, 17(11), pp.2566–2580.

Uribe, M.C. & García Alarcón, A., 2005. The ovary of viviparous teleosts. Morphological differences between the ovaries of Goodea atripinnis and Ilydon whitei (Goodeidae). In M. C. Uribe & H. J. Grier, eds. *Viviparous fishes*. Florida: New Life Publication, pp. 217–235.

Vélez-Zuazo, X. & Agnarsson, I., 2011. Shark tales: A molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). *Molecular Phylogenetics and Evolution*, 58(2), pp.207–217.

Wake, M.H., 1977. The reproductive biology of caecilians: an evolutionary perspective. In D. H. Taylor & S. I. Guttman, eds. *The reproductive biology of amphibians*. New York:

Plenum, pp. 73–101.

Wake, M.H., 1980. The Reproductive Biology of Nectophrynoides malcolmi (Amphibia: Bufonidae), with Comments on the Evolution of Reproductive Modes in the Genus Nectophrynoides. *Copeia*, 1980(2), pp.193–209.

Watanabe, A. & Onitake, K., 2002. The urodele egg-coat as the apparatus adapted for the internal fertilization. *Zoological science*, 19(12), pp.1341–1347.

Wiklund, C. & Fagerström, T., 1977. Why do males emerge before females? *Oecologia*, 31(2), pp.153–158.

Wildman, D.E., 2011. Review: Toward an integrated evolutionary understanding of the mammalian placenta. *Placenta*, 32(SUPPL. 2), pp.1–8.

Wilkins, J.F. & Haig, D., 2003. What good is genomic imprinting: the function of parent-specific gene expression. *Nature Reviews Genetics*, 4(5), pp.359–368.

Wood, A.J. & Oakey, R.J., 2006. Genomic imprinting in mammals: emerging themes and established theories. *PLoS genetics*, 2(11), p.e147.

Wooding, P. & Burton, G., 2008. *Comparative placentation: structures, functions and evolution*, Springer.

Wourms, J.P., 1981. Viviparity: The maternal-fetal relationship in fishes. *Integrative and Comparative Biology*, 21(2), pp.473–515.

Wourms, J.P., Grove, B. & Lombardi, J., 1988. The maternal-embryonic relationship in viviparous fishes. In W. Hoar & D. Randall, eds. *Fish Physiology*. New York: Academic Press, pp. 1–134.

Wourms, J.P. & Lombardi, J., 1992. Reflections on the evolution of piscine viviparity. *Integrative and Comparative Biology*, 32(2), pp.276–293.

Xavier, F., 1973. Le cycle des voies génitales femelles de Nectophrynoides occidentalis Angel, Amphibien Anoure vivipare. *Z. Zellforsch*, 140, pp.509–534.

Xavier, F., Zuber-Vogeli, M. & Le Quang Trong, Y., 1970. Recherches sur l'activité endocrine de l'ovaire de *Nectophrynoides occidentalis* Angel (Amphibien Anoure vivipare) - I. Etude histochimique. *General and comparative Endocrinology*, 15, pp.425–431.

Xie, B. et al., 2009. The evolutionary foundation of genomic imprinting in lower vertebrates. *Chinese Science Bulletin*, 54(8), pp.1354–1360.

Yamane, T. & Miyatake, T., 2010. Induction of oviposition by injection of male-derived extracts in two Callosobruchus species. *Journal of Insect Physiology*, 56(12), pp.1783–1788.

Yandell, C.A. et al., 1999. PROTEIN CHEMISTRY AND STRUCTURE : The Kangaroo Cation-independent Mannose 6-Phosphate Receptor Binds Insulin-like Growth Factor II with Low Affinity The Kangaroo Cation-independent Mannose 6-Phosphate Receptor Binds Insulin-like Growth Factor II with Low . , 274(38), pp.27076–27082.

Yu, S. et al., 2001. Increased Insulin Sensitivity in G_s α Knockout Mice. *Journal of Biological Chemistry*, 276(23), pp.19994–19998.

Yu, S. et al., 2000. Paternal versus maternal transmission of a stimulatory G-protein α subunit knockout produces opposite effects on energy metabolism. *Journal of Clinical Investigation*, 105(5), pp.615–623.

Yu, S. et al., 1998. Variable and tissue-specific hormone resistance in heterotrimeric Gs protein α-subunit (G s α) knockout mice is due to tissue-specific imprinting of the G s α

gene. *Genetics*, 95, pp.8715–8720.

Zeh, D.W. & Smith, R.L., 1985. Paternal Investment by Terrestrial Arthropods. *American Zoologist*, 25(3), pp.785–805.

Zeh, D.W. & Zeh, J. a, 2000. Reproductive mode and speciation: the viviparity-driven conflict hypothesis. *BioEssays*, 22(10), pp.938–46.

Zeh, J.A. & Zeh, D.W., 1996. The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society B: Biological Sciences*, 263, pp.1711–1717.

Zeh, J.A. & Zeh, D.W., 1997. The evolution of polyandry II: post-copulatory defenses against genetic incompatibility. *Proceedings of the Royal Society B: Biological Sciences*, 264(1378), pp.69–75.

Table 1. Imprinted genes that enhance or restrain growth (based on the review by Tycko & Morison 2002).

Gene	Expression	Effect on growth	Source
Igf2	Paternal	+	DeChiara et al. 1990
Ins1/Ins2	Paternal	+	Giddings et al. 1994; Duvillié et al. 1997; Duvillié et al. 1998
Mest/Peg1	Paternal	+	Lefebvre et al. 1998
Peg3/Pw1	Paternal	+	Li et al. 1999
Igf2r	Maternal	-	Ludwig et al. 1996
Gnas	Maternal	-	Yu et al. 1998; Yu et al. 2000; Yu et al. 2001
Tssc3/Ipl	Maternal	-	Frank et al. 2002
Esx1	Maternal	-	Li & Behringer 1998

Box 1.

Oviparity. Mode of reproduction in which propagules enclosed within an egg envelope in the female body are released to the external environment where they hatch. Oviparity can be divided in 1) ovuliparity, 2) zygoparity or 3) embryoparity if eggs are released 1) unfertilized, 2) fertilized or 3) with an embryo in an advanced developmental state.

Viviparity. Mode of reproduction characterised by internal fertilization and egg retention, in which embryos fully develop within the female reproductive tract and are released to the external environment as free living animals.

Ovoviviparity. Mode of live-bearing reproduction in which embryos develop within the eggshell, but remain inside the female reproductive tract, where they hatch before being released to the external environment. This type of reproduction has no placental connection. Embryonic nutrition is through the yolk sac, but sometimes it can be supplemented in the latest stages of development by maternal provisioning such as oophagy or histrotrophy (see below).

Lecithotrophy. Nourishment of developing embryos that relies exclusively on egg yolk (common in ovoviviparity).

Matrotrophy. Embryonic nourishment by any maternal means other than yolk, e.g. maternal secretions or nutrients supplied through a placenta. Several types of matrotrophy have been distinguished by Blackburn and co-workers (Blackburn et al. 1985; Blackburn 2015):

- Oophagy. Embryonic nourishment where embryos ingest sibling yolk or products of their dissolution.
- Embryophagy/adelphophagy. Embryonic nourishment that is characterised by cannibalism among siblings.
- Matrophagy. Characterised by the ingestion of maternal tissues by the developing embryos
- Histophagy. Type of matrotrophy where embryos ingest maternal secretions.
- Histotrophy. Embryonic nutrition based on maternal nutrients that are absorbed by the embryo through specialised structures, such as the skin or gill epithelium (Blackburn 2015).
- Placentrotrophy. Pattern of matrotrophy in which nutrients are transferred to the developing offspring by means of a placenta, which has an embryonic and a maternal component.

Embryotrophy. Embryonic nourishment where embryos have some level of control over the nutrient intake. It includes three forms of matrotrophy; embryophagy, oophagy and matrophagy.

Ovotrophy. Embryonic nutrition through egg yolk and/or uterine fluid. Takes place in the egg case.

Figure 1. Possible pathway leading to the evolution of matrotrophic viviparity and placentae from an ancestral oviparous condition. The evolution of matrotrophic viviparity and placentotrophy from oviparity was probably the result of several selective pressures acting together. Although the apparition of live-bearing reproduction had mutual benefits for mothers and their offspring, different types of conflict also must have favoured the evolution of internal fertilisation, the staggered delivery and intake of maternal resources, and the diversity of placentae.

Fig. 2. Scheme of apparition of genomic imprinting. Genomic imprinting has been documented in therian mammals, and it is known that it has not evolved in monotremes or birds. Although the possibility of the evolution of a parent-of-origin gene expression in reptiles, amphibians and fish has been poorly investigated, future research should be devoted to some of matrotrophic taxa.

Figure 1

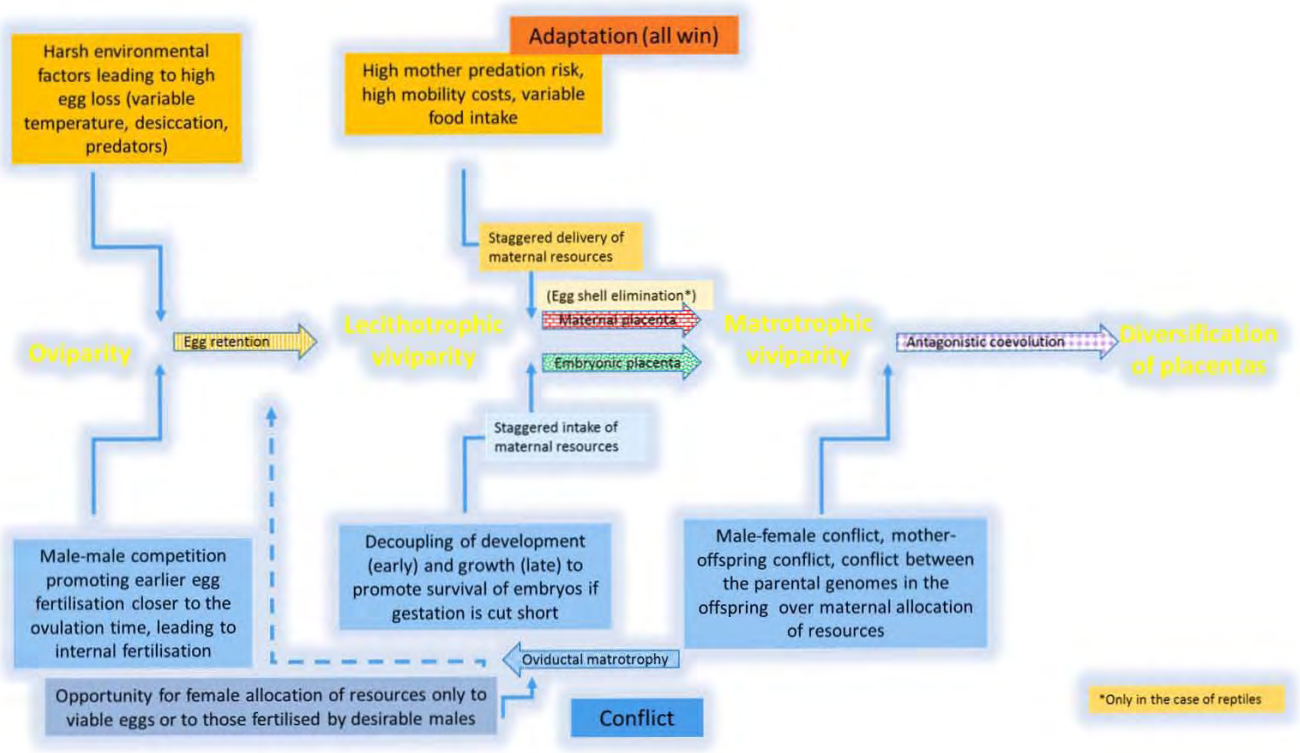
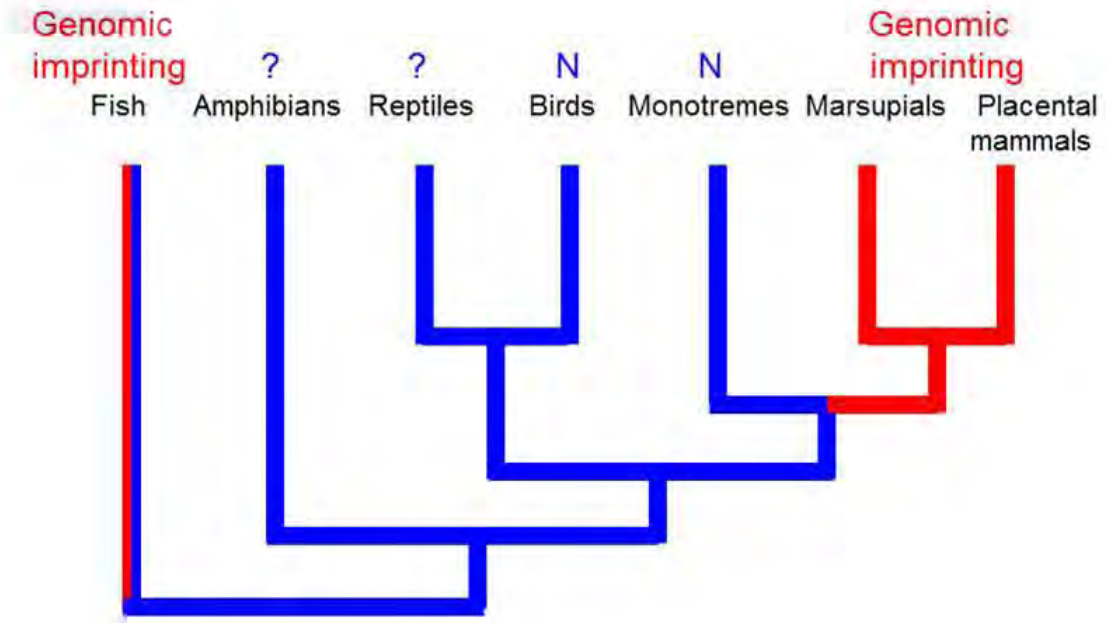

Harsh environmental factors leading to high egg loss (variable temperature, desiccation, predators)
Adaptation (all win)
High mother predation risk, high mobility costs, variable food intake
Staggered delivery of maternal resources
(Egg shell elimination*)
Maternal placenta
Embryonic placenta
Oviparity
Egg retention
Lecithotrophic viviparity
Matrotrophic viviparity
Antagonistic coevolution
Diversification of placentas
Staggered intake of maternal resources
Male-male competition promoting earlier egg fertilisation closer to the ovulation time, leading to internal fertilisation
Decoupling of development (early) and growth (late) to promote survival of embryos if gestation is cut short
Male-female conflict, mother-offspring conflict, conflict between the parental genomes in the offspring over maternal allocation of resources
Oviductal matrotrophy
Opportunity for female allocation of resources only to viable eggs or to those fertilised by desirable males
Conflict
*Only in the case of reptiles

Figure 2


Genomic imprinting
?
?
N
N
Genomic imprinting
Fish
Amphibians
Reptiles
Birds
Monotremes
Marsupials
Placental mammals

Supplementary material

Phylogenetic trees were modified from phylogenies obtained by different authors (which are indicated in the figure legends). We obtained the parenthetical notation using TreeSnatcher Plus PC and inferred the ancestral state reconstructions through parsimony using Mesquite. Trait gains were represented with figures or letters, and trait losses with a dash coloured according to the trait. Square brackets indicate that the whole group shares the gain or loss of the attribute.

We represent the modes of reproduction egg-laying (oviparous-orange ovals-) and live-bearing (ovoviviparity –blue triangles- and viviparity –red triangles-) of each taxon in the phylogenetic trees.

As we mentioned throughout the manuscript, the evolution of live-bearing reproduction led to the apparition of maternal and foetal adaptations that allowed different modes of embryonic nourishment (Box 1). According to these attributes, we categorized who holds the control over the maternal allocation of resources during embryonic development as maternal (M), maternal-embryonic (M-E), and embryonic (E) control, when these attributes allow the mother, the embryos, or both to regulate the nutrient supply and intake.

We consider as embryonic control the cases of ovoviviparity that are accompanied by oophagy, embryophagy or matrophagy. Although mothers can control the maximum number of ova or embryos they produce (maximum investment) in oophagy and embryophagic ovoviviparity (and viviparity), embryos regulate how many of them they consume. By contrast, although embryos can influence physiologically the mother into allocating more resources in species with histotrophy and histophagy, females mostly regulate the nutrient delivery according to their own interests or self-condition (Maternal control). Placentotrophy (or true vivivparity) was the only mode of embryonic nutrition that we categorized under the maternal-embryonic control category. Placentae or placenta-like structures are typically conformed by a maternal and an embryonic component whose apposition of tissues has allowed a more efficient maternal-foetal communication and nutrient transfer; however, although females can regulate the nutrient delivery, this effective physiological communication also allows embryos to deceive and manipulate females into transferring more nutrients.

Figure S1. Phylogeny of vertebrates. Viviparous taxa are shown in green. Phylogeny obtained from several sources.

Figure S2. Phylogeny of Holocephali. A dash represents the loss of the attribute and square brackets indicate a gain or loss of the trait by the whole group. Ovoviviparity and maternal control appear to be the ancestral states, which were followed by a loss of those attributes in some lineages and by a more balanced control (M-E) in the stingrays. We cannot assess whether ovoviviparity and M were lost once or twice since the phylogeny is not fully resolved. Phylogenetic tree modified from Aschliman et al. 2012.

Figure S3. Phylogeny of Elasmobranchii. A dash represents the loss of the attribute and square brackets indicate a gain or loss of the trait by the whole group. Embryonic control (E) may have been more ancient than it appears in the pgylogeny (shown with a brown rhombus), and then followed by a reversal to oviparity or by the apparition of viviparity in some lineages, which allowed females to gain subsequently control over their investment. In the rest of the clades we cannot asses whether there is an additional way of embryonic nutrition other than yolk, due to the scarcity of information. Phylogenetic tree modified from Vélez-Zuazo & Agnarsson 2011.

Figure S4. Phylogeny of fishes (Coelacanthiformes). A dash represents the loss of the attribute and square brackets indicate a gain or loss of the trait by the whole group. From this reconstruction, it seems that viviparity appeared once in the phylogeny, however, since this phylogenetic tree is very general, viviparity may have evolved in Condrychthye, Actinopterygii and Coelacanth as independent events. Coelacanthiformes is the only lineage of fish that is constituted by viviparous species. Phylogenetic tree obtained from several sources.

Figure S5. Phylogeny of Amphibians. A dash represents the loss of the attribute and square brackets indicate a gain or loss of the trait by the whole group. The evolution of internal gestation (and true viviparity) is rare among amphibians, and in most clades the evolution of ovoviviparity is very recent. Within the Caecilians ovoviviparity appears to be a common trait that evolved together with Embryonic control (E). Phylogenetic tree modified from Alexander Pyron & Wiens 2011; Kleinteich et al. 2012.

Figure S6. Phylogeny of Squamata. A dash represents the loss of the attribute and square brackets indicate a gain or loss of the trait by the whole group. The ambiguity in the data regarding viviparity did not allow to infer the sequence of events in the evolution of the diverse adaptations of live-bearing reproduction; however, it seems that once an attribute appears, additional transitions follow, and in those taxa where these

attributes are ancient, these probably led to speciation or diversification. Phylogenetic tree modified from Pyron et al. 2013.

Figure S7. Phylogeny of mammals. A dash represents the loss of the attribute and square brackets indicate a gain or loss of the trait by the whole group. Among these taxa, it seems that the maternal allocation of resources is an ancestral character and afterwards it diversified into M and M-E. Phylogenetic tree modified from The Tree of Life Web Project.

Figure S8. Phylogeny of Actinopterygii. A dash represents the loss of the attribute and square brackets indicate a gain or loss of the trait by the whole group. This phylogeny is very general, therefore, we cannot observe the sequence of the apparition of the diverse attributes; however, we can affirm that once some adaptations of live-bearing reproduction evolved, the others followed afterwards. (We traced the evolution of the attributes mentioned above in a more detailed phylogenetic tree of the viviparous lineages Antherinomorpha and Ophiidiformes). We did not find a satisfactory phylogeny of Perciformes, and we did not trace the ancestral states of traits of Signathiformes since all the species have the same attributes. Although viviparity is paternal within this last clade, which represents a role reversal, we used the same symbols for paternal and maternal control. Phylogenetic tree modified from Near et al. 2012.

Figure S9. Phylogeny of Ophidiiformes. A dash represents the loss of the attribute and square brackets indicate a gain or loss of the trait by the whole group. All the attributes related to live-bearing reproduction appeared within the family Bithitidae, thus we need a phylogeny of this taxon to elucidate the evolutionary sequence of events within this family. We propose, however, that it first appeared ovoviviparity from an oviparous ancestor, then the adaptations for M or E, and then the rest of the traits. Phylogenetic tree modified from Møller et al. 2016.

Figure S10. Phylogeny of Cyprinodontiformes. A dash represents the loss of the attribute and square brackets indicate a gain or loss of the trait by the whole group. Among Cyprinodontiformes the most diverse lineage is the one with more adaptations for internal gestation. This gives support to the proposal that a diversity of forms of nutrient transfer is linked to speciation. Phylogenetic tree modified from Parenti 2005.

Figure S11. Phylogeny of Beloniformes. A dash represents the loss of the attribute and square brackets indicate a gain or loss of the trait by the whole group. Live-bearing reproduction evolved in only one family of beloniform fishes. A phylogeny of

Zenarchopteridae would allow us to elucidate the sequence of events, however, it seems again that once one attribute evolves, the others follow afterwards. This diversity of forms of nutrient transfer apparently implies a diversity of taxa. Phylogenetic tree modified from Parenti 2005.

Figure S1

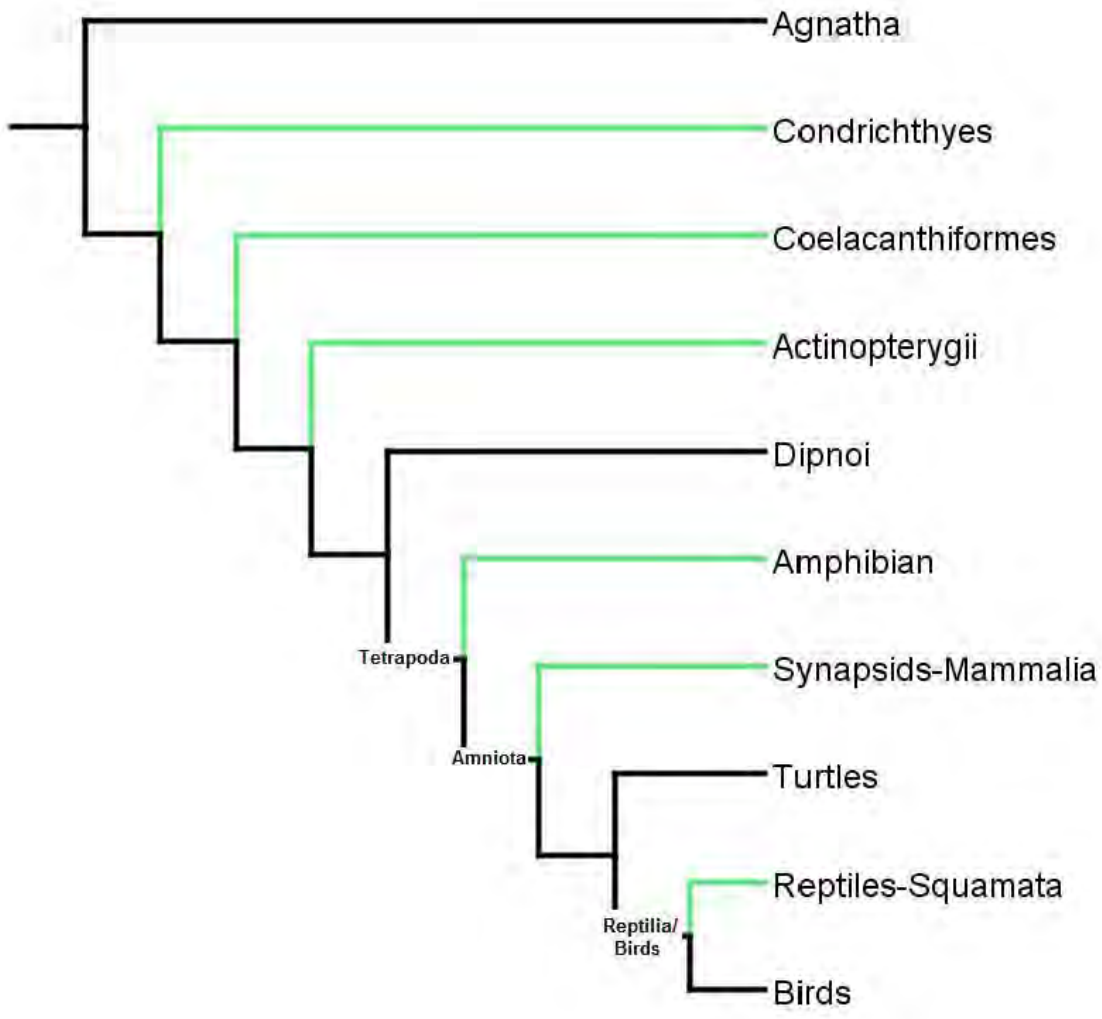

Agnatha
Condrichthyes
Coelacanthiformes
Actinopterygii
Dipnoi
Amphibian
Tetrapoda
Synapsids-Mammalia
Amniota
Turtles
Reptiles-Squamata
Reptilia/
Birds
Birds

Figure S2.

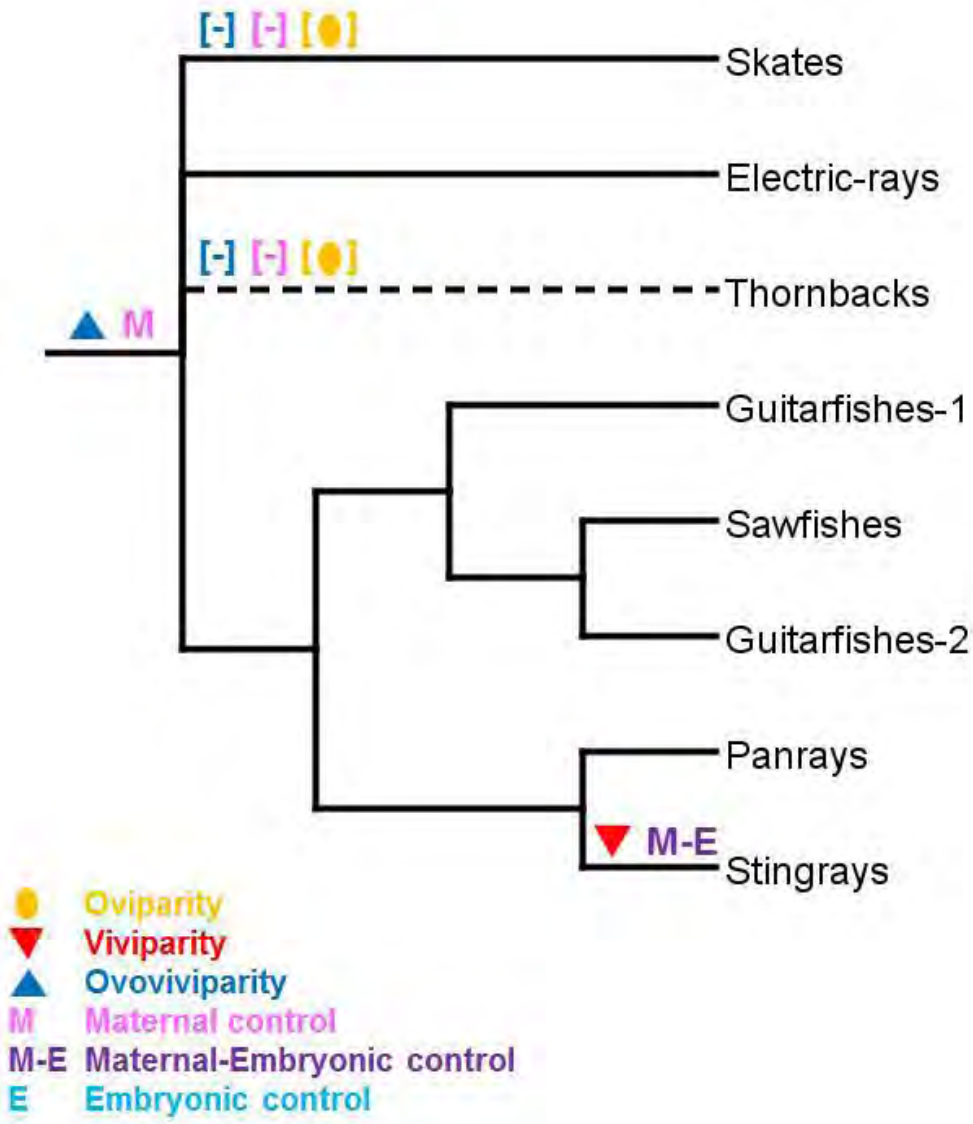

[-] [-] [●]
Skates
Electric-rays
[-] [-] [●]
Thornbacks
▲ M
Guitarfishes-1
Sawfishes
Guitarfishes-2
Panrays
▼ M-E
Stingrays
● Oviparity
▼ Viviparity
▲ Ovoviviparity
M Maternal control
M-E Maternal-Embryonic control
E Embryonic control

Figure S3.

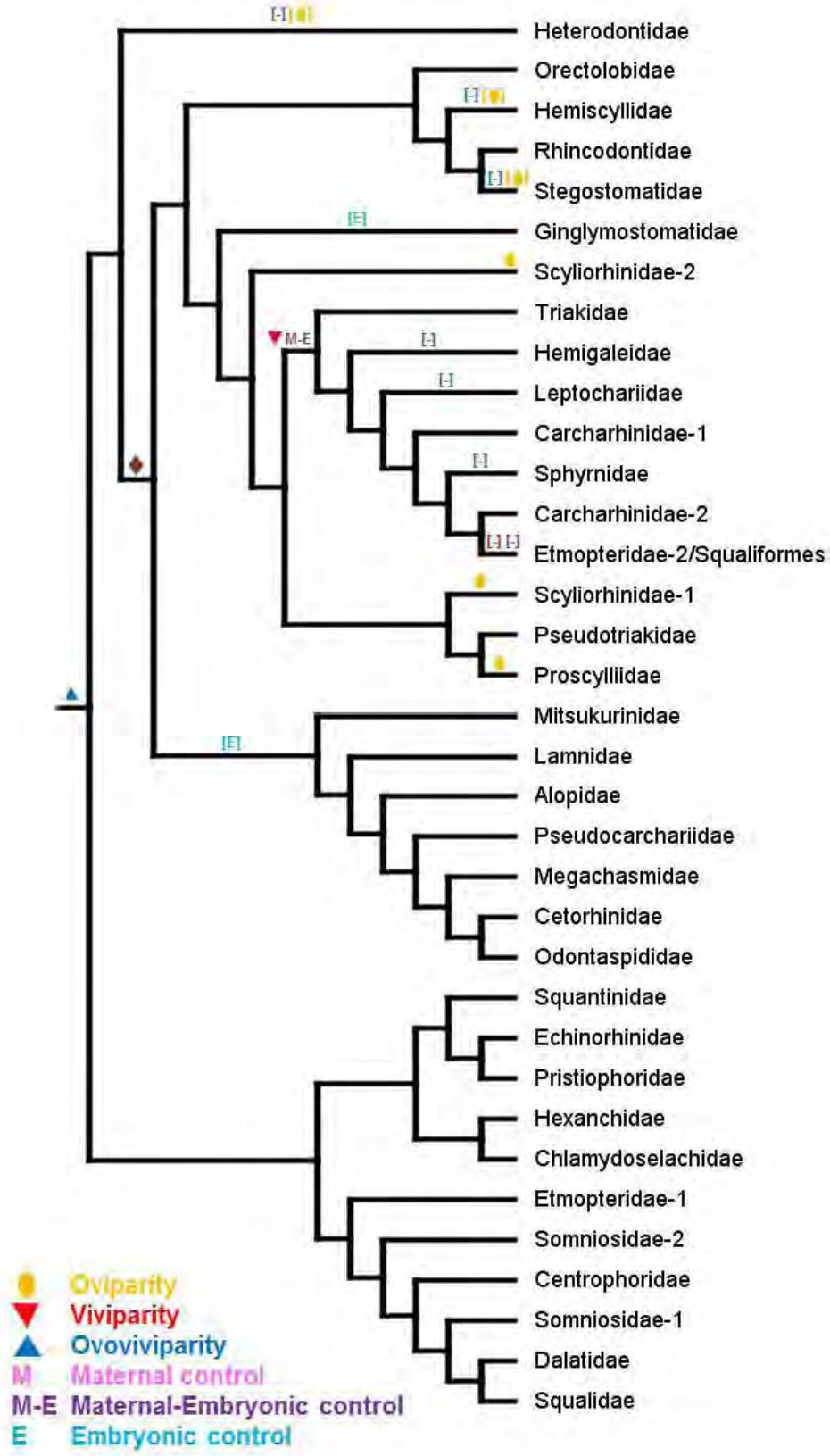

Heterodontidae
Orectolobidae
Hemiscyllidae
Rhincodontidae
Stegostomatidae
Ginglymostomatidae
Scyliorhinidae-2
Triakidae
Hemigaleidae
Leptochariidae
Carcharhinidae-1
Sphyrnidae
Carcharhinidae-2
Etmopteridae-2/Squaliformes
Scyliorhinidae-1
Pseudotriakidae
Proscylliidae
Mitsukurinidae
Lamnidae
Alopidae
Pseudocarchariidae
Megachasmidae
Cetorhinidae
Odontaspididae
Squantinidae
Echinorhinidae
Pristiophoridae
Hexanchidae
Chlamydoselachidae
Etmopteridae-1
Somniosidae-2
Centrophoridae
Somniosidae-1
Dalatidae
Squalidae
M-E
Oviparity
Viviparity
Ovoviviparity
M Maternal control
M-E Maternal-Embryonic control
E Embryonic control

Figure S4.

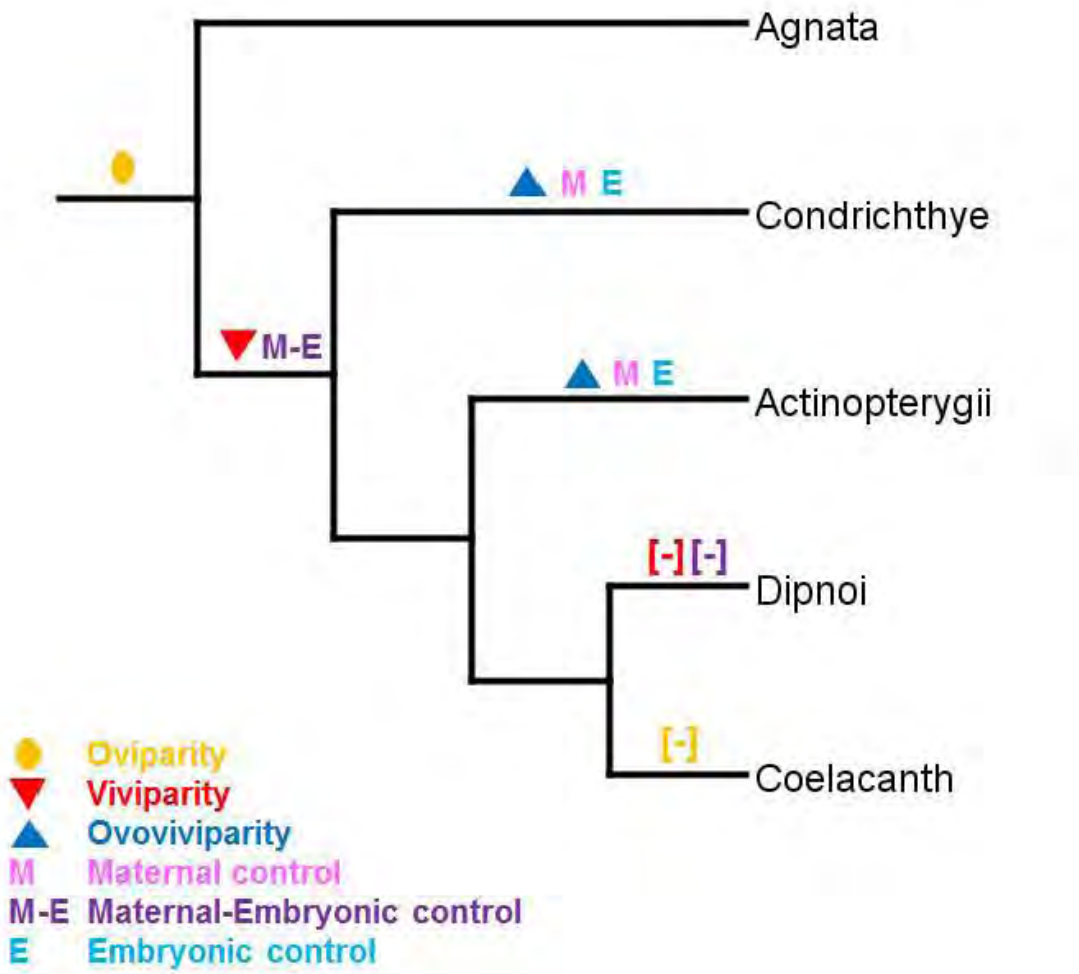

Agnata
M E
Condrichthye
M-E
M E
Actinopterygii
[-] [-]
Dipnoi
[-]
Coelacanth
Oviparity
Viviparity
Ovoviviparity
M Maternal control
M-E Maternal-Embryonic control
E Embryonic control

Figure S5.


Leiopelmatidae
Ascaphidae
Pipidae
Rhinophrynidae
Scaphiopodidae
Pelodytidae
Megophryidae
Pelobatidae
Heleophrynidae
Calyptocephalellidae
Myobatrachidae
Ceuthomantidae
Brachycephalidae
Eleutherodactylidae
Craugastoridae
Hemiphractidae
Hylidae
Bufonidae
Dendrobatidae
Leptodactylidae
Centrolenidae
Allophrynidae
Ceratophryidae
Odontophrynidae
Telmatobiidae
Batrachylidae
Rhinodermatidae
Cycloramphidae
Alsodidae
Hylodidae
Microhylidae
Brevicipitidae
Hemisotidae
Arthroleptidae
Hyperoliidae
Ptychadenidae
Micrixalidae
Phrynobatrachidae
Conrauidae
Petropedetidae
Pyxicephalidae
Ceratobatrachidae
Nyctibatrachidae
Dicroglossidae
Ranixalidae
Ranidae
Rhacophoridae
Mantellidae
Sooglossidae
Nasikabatrachidae
Bombinatoridae
Discoglossidae
Alytidae
Rhinatrematidae
Ichthyophiidae
Hynobiidae
Cryptobranchidae
Sirenidae
Proteidae
Rhyacotritonidae
Amphiumidae
Plethodontinae
Hemidactylinae
Bolitoglossinae
Spelerpinae
Ambystomatidae
Dicamtodontidae
Salamandrininae
Pleurodelinae
Salamandrinae
Scolecomorphidae
Herpelidae
Indotyphlidae
Dermophiidae
Siphonopidae
Typhlonectidae
Scolecomorphinae
Caeciliinae
Herpelinae
Oviparity
Viviparity
Ovoviviparity
M Maternal control
M-E Maternal-Embryonic control
E Embryonic control

Figure S6.

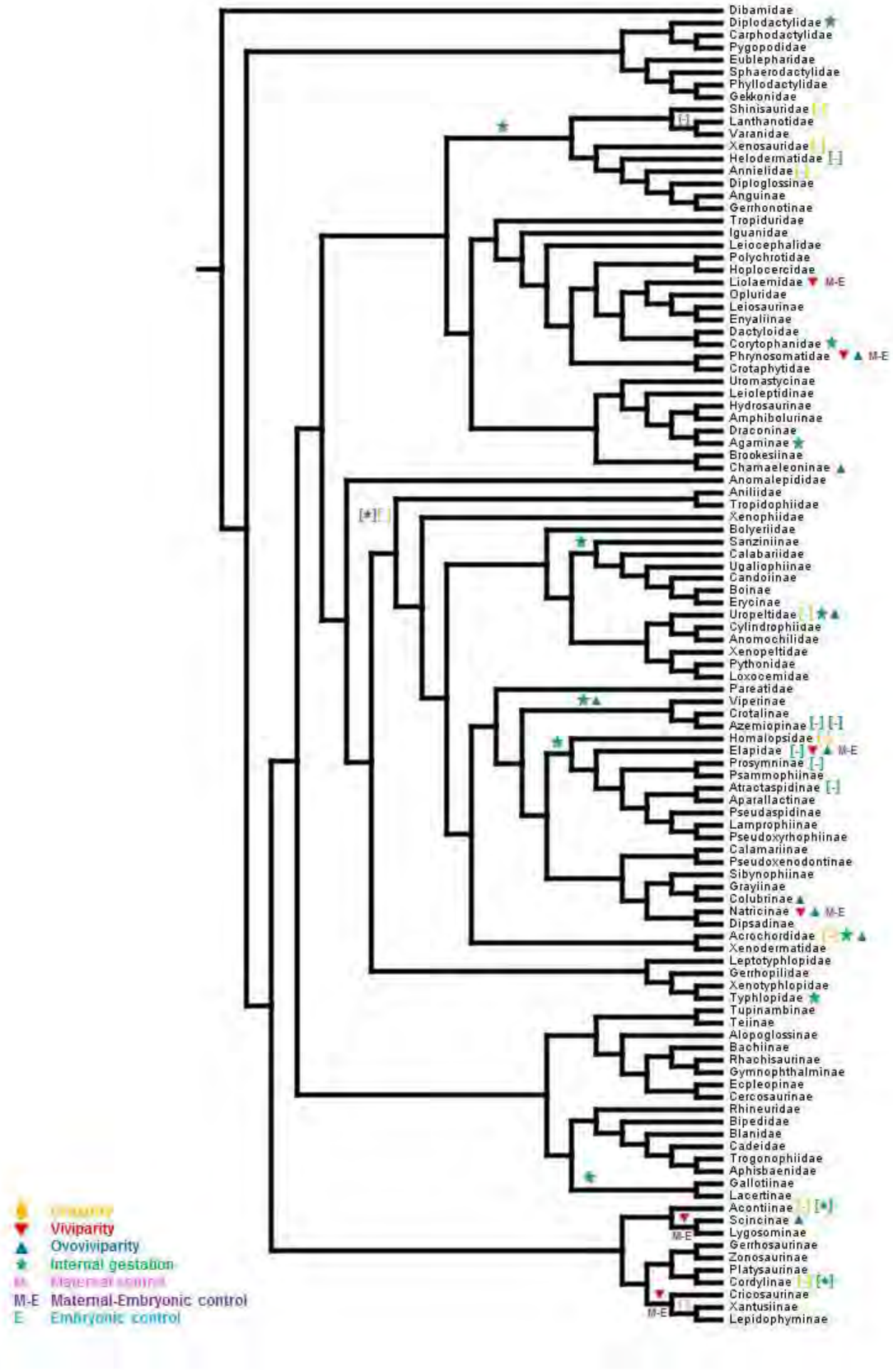

Dibamidae
Diplodactylidae
Carphodactylidae
Pygopodidae
Eublepharidae
Sphaerodactylidae
Phyllodactylidae
Gekkonidae
Shinisauridae
Lanthanotidae
Varanidae
Xenosauridae
Helodermatidae
Annielidae
Diploglossinae
Anguinae
Gerrhonotinae
Tropiduridae
Iguanidae
Leiocephalidae
Polychrotidae
Hoplocercidae
Liolaemidae
Opluridae
Leiosaurinae
Enyaliinae
Dactyloidae
Corytophanidae
Phrynosomatidae
Crotaphytidae
Uromastycinae
Leioleptidinae
Hydrosaurinae
Amphibolurinae
Draconinae
Agaminae
Brookesiinae
Chamaeleoninae
Anomalepididae
Aniliidae
Tropidophiidae
Xenophiidae
Bolyeriidae
Sanziniinae
Calabariidae
Ugaliophiinae
Candoiinae
Boinae
Erycinae
Uropeltidae
Cylindrophiidae
Anomochilidae
Xenopeltidae
Pythonidae
Loxocemidae
Pareatidae
Viperinae
Crotalinae
Azemiopinae
Homalopsidae
Elapidae
Prosymninae
Psammophiinae
Atractaspidinae
Aparallactinae
Pseudaspidinae
Lamprophiinae
Pseudoxyrhophiinae
Calamariinae
Pseudoxenodontinae
Sibynophiinae
Grayiinae
Colubrinae
Natricinae
Dipsadinae
Acrochordidae
Xenodermatidae
Leptotyphlopidae
Gerrhopilidae
Xenotyphlopidae
Typhlopidae
Tupinambinae
Teiinae
Alopoglossinae
Bachiinae
Rhachisaurinae
Gymnophthalminae
Ecpleopinae
Cercosaurinae
Rhineuridae
Bipedidae
Blanidae
Cadeidae
Trogonophiidae
Aphisbaenidae
Gallotiinae
Lacertinae
Acontiinae
Scincinae
Lygosominae
Gerrhosaurinae
Zonosaurinae
Platysaurinae
Cordylinae
Cricosaurinae
Xantusiinae
Lepidophyminae
Viviparity
Ovoviviparity
Internal gestation
Maternal control
M-E Maternal-Embryonic control
E Embryonic control

Figure S7.

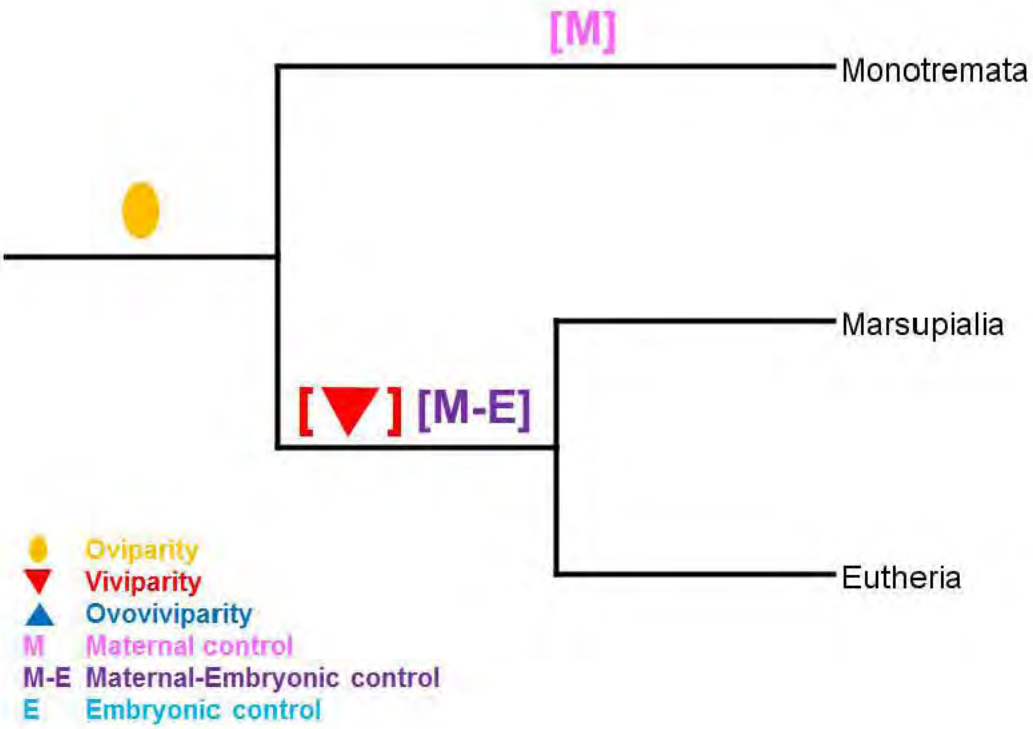

[M]
Monotremata
Marsupialia
[▼] [M-E]
Eutheria
Oviparity
Viviparity
Ovoviviparity
M Maternal control
M-E Maternal-Embryonic control
E Embryonic control

Figure S8.

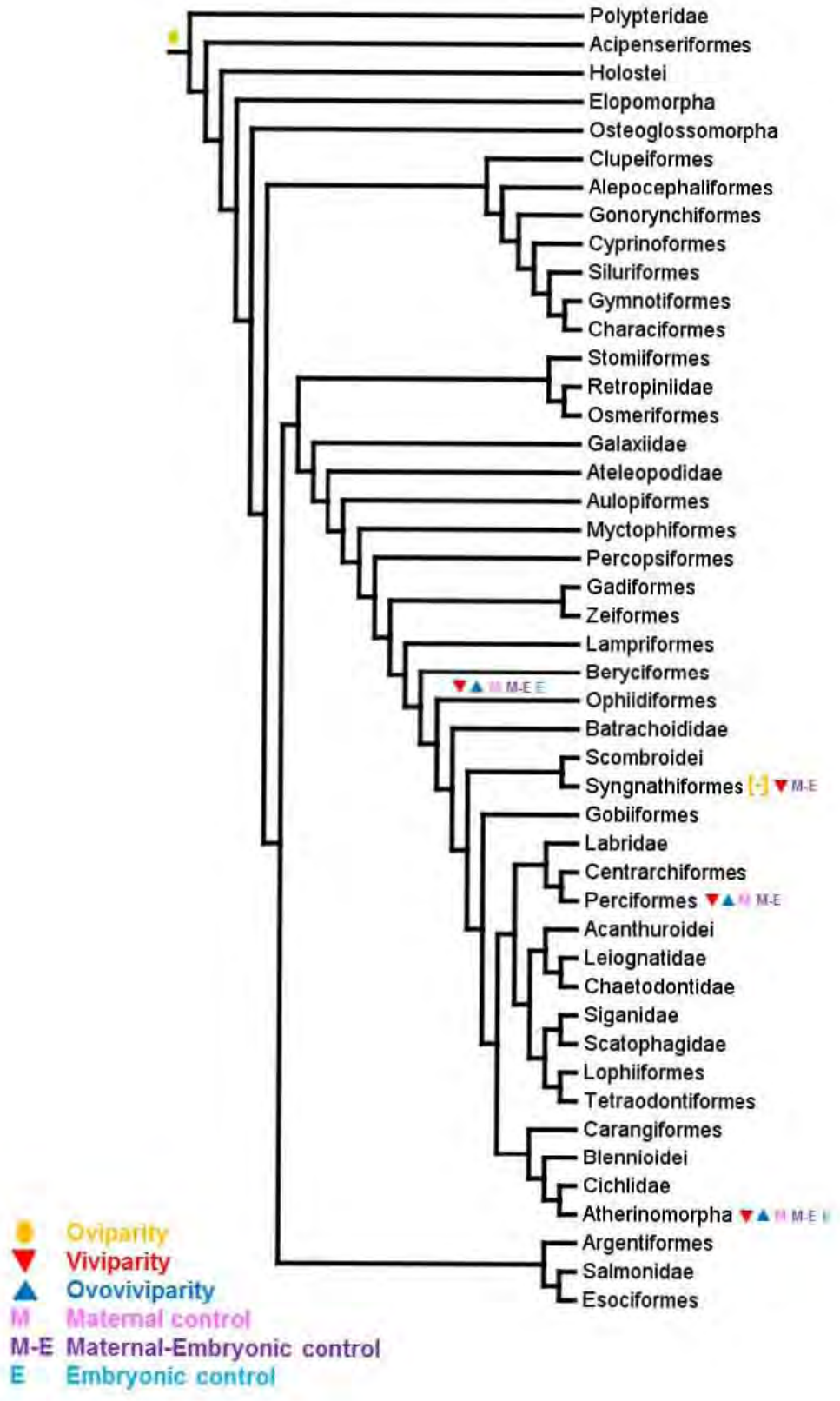

Polypteridae
Acipenseriformes
Holostei
Elopomorpha
Osteoglossomorpha
Clupeiformes
Alepocephaliformes
Gonorynchiformes
Cyprinoformes
Siluriformes
Gymnotiformes
Characiformes
Stomiiformes
Retropiniidae
Osmeriformes
Galaxiidae
Ateleopodidae
Aulopiformes
Myctophiformes
Percopsiformes
Gadiformes
Zeiformes
Lampriformes
Beryciformes
Ophiidiformes
Batrachoididae
Scombroidei
Syngnathiformes
Gobiiformes
Labridae
Centrarchiformes
Perciformes
Acanthuroidei
Leiognatidae
Chaetodontidae
Siganidae
Scatophagidae
Lophiiformes
Tetraodontiformes
Carangiformes
Blennioidei
Cichlidae
Atherinomorpha
Argentiformes
Salmonidae
Esociformes
Oviparity
Viviparity
Ovoviviparity
M Maternal control
M-E Maternal-Embryonic control
E Embryonic control

Figure S9.

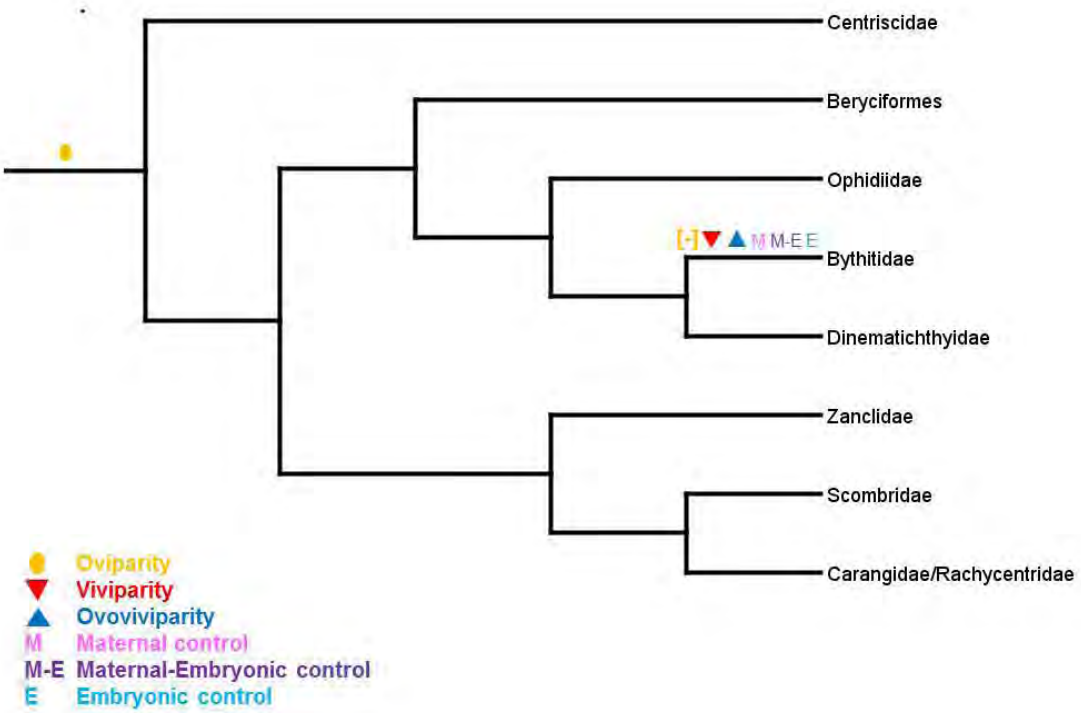

Centriscidae
Beryciformes
Ophidiidae
[-] M M-E E
Bythitidae
Dinematichthyidae
Zanclidae
Scombridae
Carangidae/Rachycentridae
Oviparity
Viviparity
Ovoviviparity
M Maternal control
M-E Maternal-Embryonic control
E Embryonic control

Figure S10.

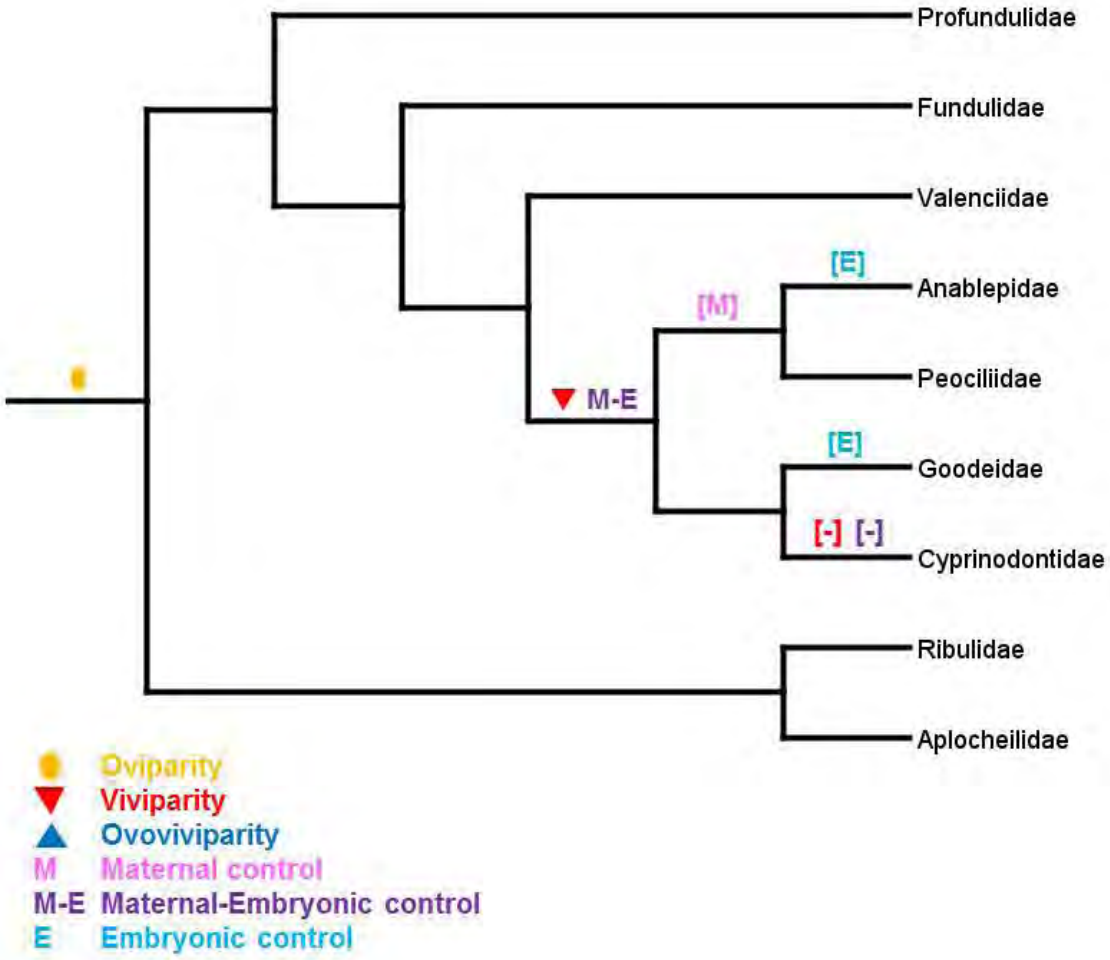
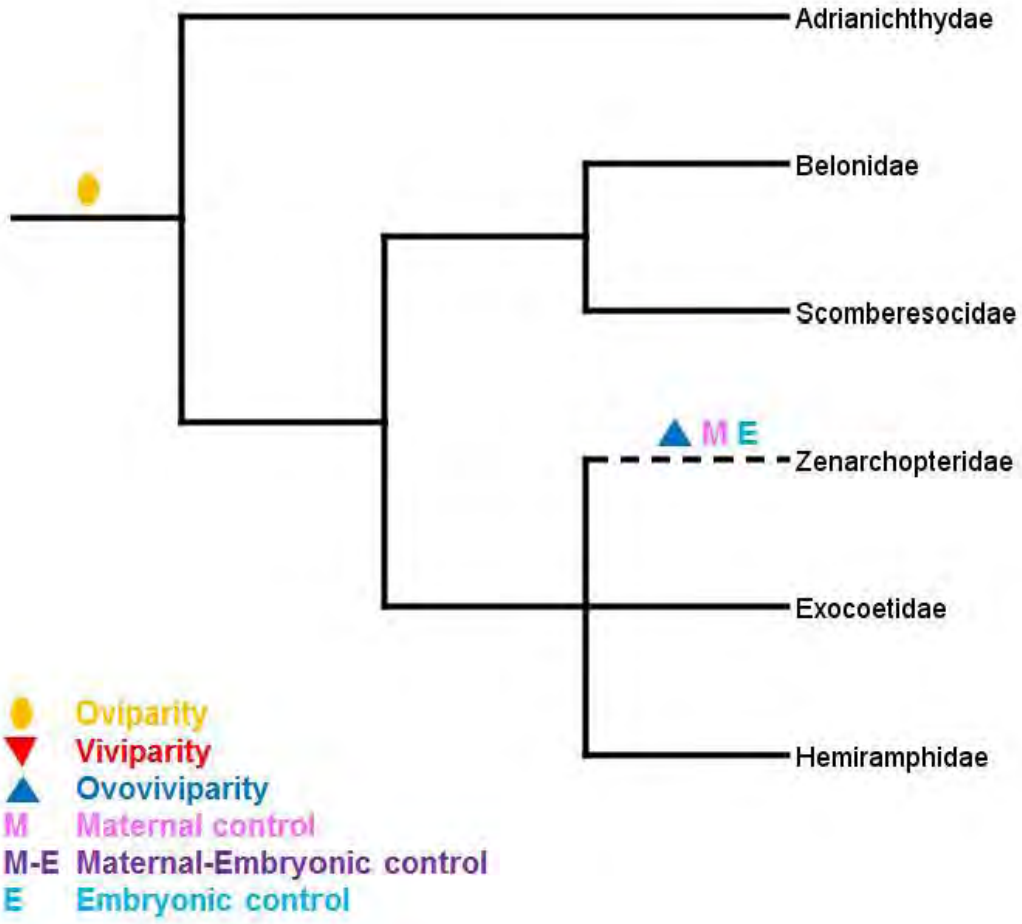

Profundulidae
Fundulidae
Valenciidae
[E]
Anablepidae
[M]
Peociliidae
M-E
[E]
Goodeidae
[-] [-]
Cyprinodontidae
Ribulidae
Aplocheilidae
Oviparity
Viviparity
Ovoviviparity
M Maternal control
M-E Maternal-Embryonic control
E Embryonic control

Figure S11.


Adrianichthydae
Belonidae
Scomberesocidae
M E
Zenarchopteridae
Exocoetidae
Hemiramphidae
Oviparity
Viviparity
Ovoviviparity
M Maternal control
M-E Maternal-Embryonic control
E Embryonic control

CHAPTER 3

The evolution of placentas in polyginous systems provided the physiological means by which males could influence maternal allocation of resources. An antagonistic coevolution between the parental genomes within the offspring apparently favoured or led the evolution of genomic imprintig in mammals, a monoallelic pattern of gene expression in which alleles are expressed in a parent-of-origin manner…

¿Qué es la impronta genética? Teorías, fundamentos y mecanismos

What is genomic imprinting? Theories, foundations and mechanisms.

Yolitzi Saldivar Lemus[1*] & Constantino Macías Garcia[1]

[1] Departamento de Ecología evolutiva, Instituto de Ecología, UNAM. A. P. 70-275, C. P. 04510, México City, México.

Manuscript

Abstract.- Genomic imprinting is a gene expression pattern in which alleles are expressed in a parent-of-origin manner. Allele silencing is achieved via DNA methylation that takes place during gamete formation in processes of global erasure and selective demethylation according to the origin of the allele. These epigenetic marks are maintained after fertilization and will be read in the somatic cells. Several theories have been proposed to explain the evolution and maintenance of genomic imprinting in eutherian mammals, marsupials and flowering plants. The Kinship Theory suggests that genomic imprinting evolved in viviparous mammals and plants as a consequence of the diverging interests of paternally inherited (padumnal) and maternally inhierited (madumnal) alleles within the offspring over maternal allocation during embryonic development. In oviparous species, maternal allocation cannot be influenced via gene expression. Nevertheless, several fish, reptiles and amphibian species have evolved the attributes of viviparity, polygamy and genes able to influence the maternal allocation of resources that, according to the The Kinship Theory, made mammals susceptible to the evolution of genomic imprinting. Therefore, we suggest that more research should be addressed to the evolution of genomic imprinting in other viviparous (and possibly more matrotrophic) taxa.

Keywords: Conflict, coadaptation, viviparity, matrotrophy, igf2.

1. Introduction

Genomic imprinting is a widespread phenomenon, in which certain genes are expressed in a parent-of-origin manner, that is, the two parental alleles of a gene are expressed differentially within an individual (Tilghman 1999). This type of epigenetic gene regulation is found in mammals (eutherians and marsupials; Constância et al. 2002; DeChiara et al. 1990; O'Neill et al. 2000) and plants (Scott et al. 1998; Lin 1984) and results in a monoallelic gene expression (in animals) or in a ploidy ratio of 2m:1p (maternal and paternal respectively) in some tissues (e.g. endosperm) in plants.

This epigenetic phenomenon is present in therian mammals, but not in monotremes or birds (O'Neill et al. 2000; J. Keith Killian et al. 2001). Genomic imprinting, although it appeared before the marsupials and eutherian split, has evolved differently in these groups. Eutherians have a larger number of imprinted genes than marsupials (Renfree et al. 2012) (Figure 1).

2. Molecular basis

Genomic imprinting involves epigenetic regulation of gene expression in which allele silencing is achieved by DNA or histone methylation (addition of methyl groups to cytosines or adenines of the DNA chain) that does not modify the DNA sequence itself (Lewin 2004). This epigenetic mark for genomic imprinting must meet some conditions: i) in diploid organisms, once it is established, it must remain on the same parental chromosome, ii) it must be established in the gametes, since alleles are in different compartments and thus can suffer differential modifications, iii) it must be stably maintained and inherited after fertilization and mitosis in all the tissues; and iii) these modifications must be capable of being de-methylated and re-methylated in order to be able to transmit this epigenetic mark the progeny (Barlow & Bartolomei 1997; Bartolomei & Tilghman 1997)

DNA silencing may be achieved via cytosine methylation in CpG islands, which are sequences conformed by repeats of CG dinucleotides (Bird 1986). The enzymes

responsible for establishing and maintaining DNA methylation are called DNA methyltransferases (DNMTs). They add methyl group to the fifth carbon of cytosine residues within the CG dinucleotides of CpG islands. These CpG-rich islands have on average a ~60% of G-C content, compared to the 40% in bulk DNA, and typically take the form of stretches of DNA of usually 1-2 kb long (Lewin 2004) that are called Differentially Methylated Regions (DMR). DMR are categorised in primary DMR when they were originated in the germline of a parent, and secondary, when they appeared later during development (e.g. after implantation; Vrana 2007).

Methylation of DNA is the key factor for imprinting because it regulates gene expression. The differential methylation pattern of each parental allele must be translated into a monoallelic gene expression. This is known as the reading of the imprint, and may involve several trans-acting factors that can detect the allele-specific methylation state and can interact with other factors in order to inhibit or start the transcription process (Constância et al. 1998).

Although DNA methylation regularly induces DNA silencing, supressed alleles are not always methylated. Methylation patterns can lead to different effects in gene expression. Several examples are described in the literature. The expression of the paternal copy of *h19,* an embryonic transcript of unknown function (Poirier et al. 1991), is supressed by DNA methylation of the promoter region (Bartolomei et al. 1991; Ferguson-Smith et al. 1993), whereas the active paternal allele of SNRPN, one of a gene family that encode proteins involved in pre-mRNA splicing, has an unmethylated transcription start site and a methylated CpG island in intron 5 (Glenn et al. 1996). In the case of *igf2r*, where the maternal allele is the active one, the methylation of an intron impedes the translation of antisense RNA that inhibits *igf2's* expression (Wutz & Barlow 1998). Gene regulation can also be linked between neighbouring genes. The expression of *igf2* and *H19* are closely related. There is CpG island (primary Differential Methylated Region –DMR-) on the intergenic region between these two

genes (Tremblay et al. 1995). In this case the maternal allele is hypomethylated which allows the CTCF protein insulators to attach to this region in order to impede the enhancer to act on *igf2's* promoter (Hark et al. 2000). Meanwhile, *H19*'s promoter is unmethylated and the enhancer can act on it, leading to H19 transcription (Ideraabdullah et al. 2008), which consequently inhibits igf2 expression (Leighton et al. 1995)(Figure 2). The paternal expressed *igf2* allele, on the other hand, has a hypermethylated CpG island that obstructs the CTCFs attachment. This allows the enhancer to reach *igf2*'s promoter for posterior expression. Such effect is enhanced by a secondary DMR that form DNA loops that facilitate the interaction between the promoter and the enhancer (Murrell et al. 2004). Furthermore, the CpG island methylation in the intergenic region extends to *H19*'s promoter leading to its ulterior silencing (Vrana 2007; Figure 2).

During gamete formation genomic imprinting marks must be removed and reset according to the specific gene and sex of the parent, thus methylation is not stable in cells across generations. During gametogenesis epigenetic modifications are removed in both chromosomes of both sexes (see Constância et al. 1998).This occurs as a result of global demethylation (which includes imprinted genes) during the early stages of germ cells formation (Brandeis et al. 1993) known as erasure (Figure 3). After erasure the process of Establishment occurs, whereby selective gene remethylation takes place via the action of DNA methyltransferase (DNmt1), which is the most important DNA methyltransferase in mouse embryos (Yoder et al. 1997) and operates in the nucleus of the oocyte and spematogonya (Mertineit et al. 1998). Although methylation of DNA is crucial for imprinting, it is not clear whether methylation is the primary imprint signal that needs to be removed and reset in the germline (see Constância et al. 1998)

Despite the importance of methylation for imprinting, gametic imprints represent only a part of the total methylation found in somatic tissues. After erasure and establishment, some of the methylation in gametic DNA is erased again during preimplantation

development and is restabished after implantation (see Constância et al. 1998). Indeed, the higher level of methylation in the paternal allele of *H19* is established during postimplantation in mouse embryos (Tremblay et al. 1997). Additionally, some DMRs show tissue-specific methylation patterns (Moore et al. 1997; Feil et al. 1994) although once somatic tissues are established in the embryonic phase, allelic methylation patterns seem to be relatively stable (Eversole-Cire et al. 1993). Once fertilization takes place and during postimplantation development, the allelic expression according the imprinting marks will probably remain during offspring's development and/or life.

3. Characteristics of imprinted genes

It is difficult to state the characteristics of imprinted genes, however Vrana (2007) has synthesised the most important generalizations about these genes:

1. Most imprinted genes mainly affect prenatal and neonatal growth.
2. Most imprinted genes are expressed in extraembryonic tissues.
    a) Imprinted genes are found in clusters, which seems to be a result of a coevolutionary gene expression produced by a beneficial combination of alleles that were favoured by selection (Wolf 2013). Additionally, these clusters tend to contain both maternally and paternally expressed genes.
3. Imprinted domains have at least one region where the two parental alleles differ in the amount of DNA methylation.
4. Maternally and paternally expressed genes tend to have antagonistic effects.
5. Many imprinted genes do not appear to encode proteins.

Imprinting is not always stable, genes may be imprinted only in certain tissues, at certain times during development (Yuan et al. 2011), and imprinting may be incomplete, that is,

allelic expression may be biased against, but not completely absent in the “silent” allele (Vrana 2007; see Sato & Stryker 2010).

4. **Evolution of genomic imprinting: Different Theories**

4.1. DNA silencing and genomic imprinting: from host defence to gene regulation

Cells have always been exposed to foreign DNA, and therefore it have been suggested that eukaryotic and prokaryotic cells have developed several mechanisms against the uptake, integration, and expression of foreign DNA, which includes DNA methylation (Doerfler 1991). One of the attributes of DNA methylation that possibly made it the most efficient mechanism in prokaryotes and eukaryotes for defence against foreign DNA is that is reversible (Bestor 1990; Doerfler 1991). This characteristic allows genes or alleles whose products are advantageous for the transformed cells to be selectively excluded from this silencing mechanism (Doerfler 1991).

Barlow (1993) proposed the host defence theory to explain the evolution of imprinting as an extension of the host defence role that DNA methylation plays against invading organisms or foreign DNA. This implies that genomic imprinting originated from a system that served to neutralize foreign DNA, where the imprinting factor (methylation) modifies the imprinting box (a DNA sequence similar to that of a foreign agent), and thus silences transcription. According to this theory, this modification is done in a parent-specific manner during gametogenesis.

4.2. The necessity of a monoallelic gene Expression mechanism: Complementation hypothesis

Biallelic gene expression bring some advantages such as the minimisation of the occurrence of recessive genetic disorders; nonetheless, the evolution of genomic imprinting cause a monoallelic expression of genes in biallelic organisms. The complementation hypothesis (Kaneko-Ishino et al. 2003) states the necessity a monoallelic expression of the genes that evolved imprinting.

Pegs and Megs (Paternally Expressed Genes and Maternally Expressed Genes, respectively) are spread all over the mammalian genome and many of them are essential for development. Under the scenario where sexual conflict was the force that imposed selection pressures on gene expression resulting in the evolution of imprinting, then this theory predicts the evolution and maintenance of complementation between paternal and maternal genomes becomes necessary for a normal functioning (Kaneko-Ishino et al. 2006). This is thought to have extended to other genes whose expression is linked to the first imprinted ones, resulting in the formation of clusters with a coupled regulation.

4.3. A coadaptation strategy between mother and offspring: Coadaptation hypothesis

The coadaptation hypothesis suggests that the exclusive expression of maternal alleles is favoured by natural selection because it increases the adaptive integration of offspring and maternal genomes, leading to higher offspring fitness (Wolf & Hager 2006; Wolf & Brodie III 2009). This suggests that genomic imprinting coadaptatively regulates mammalian embryonic development, maternal care and reproductive behaviour (Curley et al. 2004; Keverne & Curley 2008).

The most representative example that fits the predictions of this theory is the one of *Peg3*. This gene is imprinted in 3 tissues: the foetal placenta, the foetal hypothalamus, and the maternal hypothalamus (Renfree et al. 2009). *Peg 3* is paternally expressed in the brain and in the placenta and is essential for normal pup suckling behaviour. It is also associated with the size of the placenta, foetal and postnatal growth, weaning age and puberty onset, maternal care, maternal food intake during pregnancy, and impaired milk let-down (Curley et al. 2004). Curley and co-workers (2004) demonstrated that when the paternal copy of this gene is knocked-out, either in the offspring or in the mother, the normal development and suckling behaviour of the offspring and the maternal care are disrupted.

According to the coadaptation theory (Curley et al. 2004), offspring carrying good maternal nurturing genes will be both well provisioned and genetically predisposed towards

good mothering, and these characteristics that are a product of a monoallelic expression would become fixed easily and spread over the population, due to their beneficial effects on the fitness of individuals (Keverne & Curley 2008; see Renfree et al. 2009)

4.4. The diverging interests of paternally-inherited and maternally- inherited genes : The Kinship Theory

Trivers (1974) was the first to mention the possibility that conflict exists between parents and their offspring due to differing genetic interests, which could drive offspring to employ physiological weapons to manipulate maternal investment.

The kinship theory (Haig, 1996; More and Haig, 1991) suggests that in a polyandrous system, a conflict would arise between paternal and maternal genes over the maternal resource allocation during embryos' development. Haig and Westoby (1989) proposed that when embryos or litters are from different fathers, embryos compete with each other for resources. This may lead to an arm race between the parental alleles of genes that control maternal supply of resources. In a scenario where conflict is related to maternal allocation of resources, this theory predicts that the genes in the embryo that increase nutrient demand will be paternally expressed and the genes that inhibit growth will be maternally expressed (Haig & Westoby 1989; Haig 1996). Furthermore, the evolution of such genes will be driven by antagonistic coevolution (Chapman, 2006) toward the optimal fitness of each sex. This is the reason why genetic conflict has been considered as a tug-of-war (Moore & Haig 1991).

The evolution of genomic imprinting as a result of conflict, however, is restricted to groups that meet 3 conditions: 1) Offspring are from more than one father, among litters or within the same litter, 2) The costs derived from offspring nurishent fall preferentially on the mother, and 3) there are genes in the offspring that can influence maternal investment (Wilkins & Haig 2003). More precisely, we would expect the evolution of imprinting of

“manipulative” genes in the offspring of viviparous, polyandrous animals (or plants) where there exists a physiological maternal-foetal communication mediated by hormones.

The first evidence of conflict related to mating systems was discovered by conducting reciprocal crosses between two species the genus *Peromyscus*. Since conflict is stronger in polygamous species, when a female of the polyandrous subspecies *Peromyscus maniculatus* (deer mouse) mated with a male of the monogamous subspecies *Peromyscus polionotus* (oldfield mouse), neonates were smaller than both parental strains (Dawson 1965). When the opposite cross was obtained, offspring were considerably larger than any of the progenitors (Dawson et al. 1993) and placentas were 5-6 times greater in mass than those of the opposite cross (Rogers & Dawson 1970). These phenotypic data preceded the discovery of parent-of-origin gene expression as a consequence of the antagonistic interest of the parental genomes within the developing offspring, among which *igf2* and *igf2r* are completely described.

4.4.1. The evolution of genomic imprinting of *Igf2* and *igf2r*

The classical example that supports the kinship theory for the evolution of genomic imprinting is the one of the Insulin-like Growth Factor 2 (*igf2)* and its receptor (*igf2r*). IGF2 is a protein that promotes growth and cellular differentiation during development (Cohick & Clemmons 1993). In mammals it also regulates the placental supply of maternal nutrients, and the genetic demand of nutrients by the foetus (Constância et al. 2002). Its over expression has been related to some overgrowth disorders in humans (Morison & Reeve 1998). In contrast, *igf2r* encodes a membrane protein (cation independent mannose-6-phosphate receptor) that captures and transports the excess of IGF2 to the lysosomes for posterior degradation (Kornfeld & Mellman 1989), and it is therefore essential for regulating normal foetal growth, circulating levels of IGF2, and heart development (DeChiara et al. 1991; Lau et al. 1994).

In mouse embryos these two genes have opposite parental imprinting. While i*gf2* is paternally expressed (DeChiara et al. 1990), *igf2r* is only maternally transcribed (Barlow et al. 1991), and the disruption of the imprinting status of any of these genes can lead to growth abnormalities. DeChiara and coworkers (1990) disrupted by gene targeting one of the igf2 alleles in cultured mouse embryonic stem cells and constructed chimaeric animals. When the paternal *igf2* allele was knocked-out in mouse embryos, the mice were 40% smaller than the wildtype and that deficiency was maintained for at least 30 days post-partum. However, when the maternal allele is knocked-out there is no effect on the phenotype. In contrast, mouse mutants that inherited a targeted disruption on the imprinted *igf2r* maternal allele exhibited an increase of 135% of the normal birth weight (Ludwig et al. 1996).

The evolutionary explanation for the opposite expression pattern of these two antagonistic genes was proposed by Moore and Haig (1991). They suggested that when a mutation on a paternally expressed locus that allows to its bearer a more efficient extraction of nutrients from the mother evolves, it will have a selective advantage over the other alleles and will become fixed. As a consequence, a maternally expressed allele that can reduce those demands will develop as a counter-adaptation.

Under this scenario of antagonistic coevolution, once the paternal *igf2* overexpression is fixed, the silencing of maternal allele follows. Nonetheless, since *Igf2* imprinting appears insufficient to balance the optimal IGF2 production for females, an active site for IGF2 evolves in the cation-independent mannose-6-phospate receptor (CI-MPR, also known as *igf2r*; Morgan et al. 1986; Sara & Hall 1990) as a response to excess of paternally circulating IGF2 (Haig and Graham, 1991).

The evolution of a mutation on a gene serving to increase maternal allocation is equally possible in both parental alleles. However, when the fitness consequences differ according to the parental origin of the allele, the probability being fixed is biased towards one allele. A mutation on the paternal allele that increases IGF2 production would be

beneficial to both its carrier (the embryo) and its originator (the father), while a maternal allele that promotes *igf2* over-expression benefits only the carrier and imposes a cost on the fitness of the mother and her future offspring. On the contrary, IGF2R production exclusively increases the fitness of the mother and her descendants, thus, it is expected that selection will favour the silencing of paternal allele.

As predicted by the conditions stipulated by Wilkins and Haig (2003), *Igf2* is imprinted in eutherians and marsupials, but not in monotremes or birds due to the absence of placental development (DeChiara et al. 1991; J. Keith Killian et al. 2001; Nolan et al. 2001). Similarly *igf2r* is imprinted in marsupials and most eutherians (excluding Scandentia, Dermoptera and Primates; Barlow et al. 1991; J Keith Killian et al. 2001), but not in birds, monotremes, primates or amphibia (Killian et al. 2000; Clairmont & Czech 1989), since females of these groups lay eggs and maternal investment cannot be modified after the shell is put on. The evolution of a binding site in IGF2R of therian mammals that recognises and capture the excess of IGF2 (Dahms et al. 1993; Yandell et al. 1999), supports the Kinship Theory, where the evolution of IGF2R counteracted the negative effects of an excess of IGF2 (Haig & Graham 1991).

4.4.2. The conflict between sexes: A sexual conflict theory of genomic imprinting?

Whenever the interests of individuals differ or their optima cannot simultaneously be realised, conflict is expected to arise (Arnqvist & Rowe 2005). Conflict has been documented typically between males and females (sexual conflict; Arnqvist & Rowe 2005) and between parents and offspring (parent-offspring conflict; Trivers 1974).

Parent-offspring conflict related to parental investment is strong and has been broadly described (Trivers 1974; Crespi & Semeniuk 2004; see Schrader & Travis 2008). In viviparous systems, because of the differing genetic interests of parents and their progeny, selection could drive offspring to employ physiological weapons to manipulate maternal investment. As Dawkins noted in the selfish gene (1989) however, if there is conflict among

parents and their offspring who share half of their genome, then this conflict should be much more intense between mating partners who are not related to each other.

Sexual conflict has been widely reported in many groups of animals (Lessells & McNamara 2012; Okada et al. 2014; Osorno & Székely 2004; Macías-Garcia & González-Zuarth 2005), and plants (Haig & Wilczek 2006; Cox & Calsbeek 2009) in relation to different aspects of mating behaviour (Parker 2006).

When sexual conflict arises, we expect adaptations and counter-adaptations to evolve in both sexes as they battle for control. Such adaptations can evolve in the form of physical traits, e.g. structures for mating and fertilization (Arnqvist & Rowe 2002; Knowles & Markow 2001), or as macromolecule production such as proteins, or simple RNA transcripts (see Rice 1998; Haig 2004).

Haig (2000) proposed the Kinship Theory to explain why in biallelic organisms some alleles are expressed in a parent-of-origin manner. He suggested that since the parental genes in the offspring have diverging interests with respect to how much mothers should invest in offspring, this conflict led to a different expression pattern depending on the allele's parental origin in the previous generation.

Haig (2014) emphasised that conflict between genes of maternal and paternal origin is not the same as conflict between mothers and fathers, nevertheless, the former can be extrapolated to the latter. A gene, such as *igf2*, that encodes a protein that controls the demand for nutrients by the embryo, and the maternal nutrients supplied by the placenta, can influence mothers to invest more than their optimum when overexpressed. Since this would have a positive impact on paternal fitness and negatively impact on maternal fitness, the conflict could become more complex, involving more genes and resulting in antagonistic coevolution between the sexes (as well as mother and offspring) regulated at the genomic level.

That is apparently the case of *igf2* and *igf2r*, where the evolution of an active site on the receptor CI-MPR seems to be a maternal counter-adaptation for controlling the excess of paternal IGF2 in a process of Interlocus Contest Evolution (ICE; Rice & Holland 1997). ICE occurs when there is coevolution in which different loci within a genome interact antagonistically, resulting in a Red Queen Process. Under this scenario, this type of arms race could have given rise to the evolution of genomic imprinting of at least some genes. Although selection acts on gene expression as the consequence of diverging evolutionary interest between padumnal and madumnal alleles (paternally- and maternally-derived genes in offspring respectively), as Haig noted, the evolution of genomic imprinting can also be considered the outcome of an ICE, where the conflict between the sexes (or even among the three, mother, father and offspring) affects the other's fitness in a coevolutionary process with antagonistic effects that is regulated epigenetically.

4.5. Genomic imprinting and coadapted gene expression

Imprinted genes are usually found in clusters (Verona et al. 2003), and the pattern of imprinting is typically shared among them. Many imprinted genes seem to be interconnected through interactions mediated by proteins, RNA and DNA in a network whose interactome appears to be intolerant to errors (Sandhu 2010).

Wolf (2013) proposed that genomic imprinting of genes within a cluster evolved to coordinate coadapted gene expression, that is, once genomic imprinting was established in one gene, the other genes evolved imprinting as a coevolutionary response to match the expression pattern of their interacting partners. This pattern of expression is species-specific but can be broken down in hybrids where aberrant expression levels can lead to the loss of imprinting of more than one gene (Wolf et al. 2014).

This theory emphasises that in spite of what evolutionary force originated genomic (conflict between the padumnal and madumnal alleles over maternal investment; Haig 2000; Wilkins & Haig 2001, a processes of coadaptation between the parental and the offspring

genomes; Wolf & Hager 2006, or sexually antagonistic selection on sexually selected traits; Day & Bonduriansky 2004), the disruption of the imprinting status or the deletion of a section of one imprinted gene, may subsequently affect the expression of the other interacting genes (Leighton et al. 1995).

5. Viviparity and genomic imprinting

Viviparity is a mode of reproduction in which eggs are fertilized and retained inside the female reproductive tract where they develop. Offspring are released as completely developed and sometimes independent organisms (Patzner 2008). Viviparity has evolved over 160 times among animals including fish, amphibians, mammals, squamate reptiles and some invertebrates, such as some coelenterates, molluscs and arthropodes (Clutton-Brock 1991; Blackburn 1999; Blackburn 2015). Such live-bearing reproduction has been very successful because it favours offspring survival. Viviparity can allow offspring to be free-living when they are released into the external environment, because they developed sufficiently in a secure and stable environment with a constant supply of nutrients by the egg yolk (Lecithotrophic viviparity: Wourms 1981) or by the mother (matrotrophic viviparity; Wourms 1981).

Matrotrophic nutrition (maternal provisioning of resources in addition to the yolk) is possible without the existence of a placenta, such as in histotrophy or histophagy (see Blackburn 2015). However, the evolution of the placenta allowed a better maternal-foetal communication through a more effective gas exchange, waste elimination and maternal provisioning during embryonic development in several animal groups.

Placentation, in spite of being considered as a characteristic trait of mammals, has evolved independently in several animal groups. Placenta or placenta-like structures are found in other taxa such as scorpions, several groups of fish, reptiles and plants (Amoroso 1968; Renfree 1982; Lombardi & Wourms 1985a; Reznick et al. 2002; Blackburn 1999; Haig & Westoby 1991). One remarkable example is the placenta-like structure of goodeid fish,

called the trophotaenial placenta (Lombardi & Wourms 1979). This organ consists of external extensions of the hindgut (embryonic component) which develop in apposition with the ovarian lining (maternal component) during gestation (Lombardi & Wourms 1985b; Lombardi & Wourms 1985a). This organ allows a very effective nutrient transfer (Wourms 1977), that can increase embryonic dry weight by up to 38,700% (Zoogoneticus quitzeoensis; Wourms et al. 1988; Hollenberg & Wourms 1995)(Lombardi & Wourms 1979; Lombardi & Wourms 1988).

Although the primary function of nutrient transfer and waste elimination of the placenta is highly conserved among vertebrates, placentas show a great interspecific variation in morphology and physiology (Faber et al. 1992; Mossman 1991). The basic hormones involved in regulation of reproductive processes are highly conserved among vertebrates, but the sources, functions, and targets of these hormones differ among taxa. Crespi and Semeniuk (2004) predicted that the placental traits that vary the most among taxa will be those related to control over nutrient transfer because of differences in maternal –foetal antagonistic coevolution.

Consistently with the ideas of Crespi and Semeniuk (2004), most imprinted genes are expressed in the placenta (Kaneko-Ishino et al. 2003), and some of them are essential for placental development and growth. Among these genes, the ones that increase embryonic growth are usually paternally expressed and the genes that tend to restrict growth are maternally expressed (Renfree et al. 2012), as predicted by the Conflict Theory (Haig 2000a).

With the evolution of matrotrophic viviparity, conflict is expected to arise between mothers and embryos, sibling embryos in the womb, and maternal and paternal genomes within individual embryos. Such intra- and intergenomic conflicts result in perpetual antagonistic coevolution, thereby accelerating interpopulation postzygotic isolation and

diversifying maternal-foetal interactions (see the viviparity-driven conflict hyphothesis; Zeh & Zeh 2000)

The evolution of genomic imprinting as a result of conflict between mothers and embryos, between parental alleles or between the sexes over maternal allocation of resources has been extensively studied in mammals. However, viviparity and maternal provisioning during pregnancy, which are the main enhancers of this type of conflict, has been also documented in Chondrichthyans (sharks and rays), Osteichthyans (Coelacanths and some other teleost fish), amphibians (including salamanders, some frogs and caecilians) and squamate reptiles (such as many lizards and snakes; Blackburn 1999; Blackburn 2015; Figure 4). Additionally, although *igf2* is not imprinted in the placental fish *Heterandria Formosa* (Lawton et al. 2005), a CpG island on *igf2* of goldfish gametes showed parent-specific methylation, which suggests that the foundations of genomic imprinting are present in vertebrates other than mammals (Xie et al. 2009), and there is evidence that strongly suggest that genomic imprinting appeared in another viviparous matrotrophic fish (Saldivar Lemus et al. 2017) . Taken together, this implies that the evolution of genomic imprinting may be more widespread in the vertebrates than we are currently aware and therefore more research should be devoted to the study of conflict/coadaptation and genome evolution in these this taxa.

6. Conclusions and future directions

Genomic imprinting is a widespread phenomenon among mammals that regulates the expression of genes that encode some proteins and hormones that are essential to embryonic development. There are multiple evolutionary forces (conflict- between sexes, parental alleles, or mother and offspring- or coadaptation or a combination of both) that can drive the evolution of this parent-of-origin expression pattern. It seems however that once fixed at one locus, the pattern of expression coadaptatively spreads to the interacting genes. Although thus far the evolution of genomic imprinting seemed to be exclusive to Therian

mammals, placental viviparity and other traits thought to represent the foundations of genomic imprinting are present in fish and reptiles. Genomic imprinting is absent in poeciliids, but seems to be present in another fish family, thus, it could have evolved in other viviparous fish or reptiles, or it could at a “starting” point during its evolution. Therefore, more research on a parent-of-origin gene expression or on parent-of-origin methylation pattern should be addressed in these groups.

Acknowledgements

We thank Rebecca Boulton for her commets on the content and the English of this manuscript.

References

Amoroso, E.C., 1968. The Evolution of Viviparity. *Proceedings of the Royal Society of Medicine*, 61(11 Pt 2), pp.1188–1200.

Arnqvist, G. & Rowe, L., 2002. Antagonistic coevolution between the sexes in a group of insects. *Nature*, 415, pp.787–789.

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

Barlow, D.P., 1993. Methylation and imprinting - from host defense to gene-regulation. *Science*, 260(5106), pp.309–310.

Barlow, D.P. et al., 1991. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature*, 349, pp.84–87.

Barlow, D.P. & Bartolomei, M.S., 1997. Genomic Imprinting in Mammals. *Annu. Rev. Genet.*, 31, pp.493–525.

Bartolomei, M.S. & Tilghman, S.M., 1997. Genomic Imprinting in mammals. *Annual Review of Genetics*, 31, pp.493–525.

Bartolomei, M.S., Zemel, S. & Tilghman, S.M., 1991. Parental imprinting of the mouse H19 gene. *Nature*, 351(6322), pp.153–5.

Bestor, T.H., 1990. DNA Methylation: Evolution of a Bacterial Immune Function into a Regulator of Gene Expression and Genome Structure in Higher Eukaryotes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 326(1235), pp.179–187.

Bird, A.P., 1986. CpG-rich islands and the function of DNA methylation. *Nature*, 321(6067), pp.209–213.

Blackburn, D.G., 2015. Evolution of vertebrate viviparity and specializations for fetal nutrition: A quantitative and qualitative analysis. *Journal of Morphology*, 276(8), pp.961–990.

Blackburn, D.G., 1999. Viviparity and oviparity: Evolution and reproductive strategies. *Encyclopedia of Reproduction*, 4, pp.994–1003.

Brandeis, M. et al., 1993. The ontogeny of allele-specific methylation associated with imprinted genes in the mouse. *The EMBO journal*, 12(9), pp.3669–3677.

Clairmont, K.B. & Czech, M.P., 1989. Chicken and Xenopus Mannose 6-Phosphate Receptors Fail to Bind Insulin-like Growth Factor II. *Journal of Biological Chemistry*, 264(28), pp.16390–16392.

Clutton-Brock, T.H., 1991. *The Evolution of Parental Care* J. R. Krebs & T. H. Clutton-Brock, eds., New Jersey: Princeton University Press.

Cohick, W.S. & Clemmons, D.R., 1993. The Insulin-Like Growth Factors. *Annual Review of Physiology*, 55(1), pp.131–153.

Constância, M. et al., 1998. Imprinting mechanisms. *Genome Research*, 8, pp.881–900.

Constância, M. et al., 2002. Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature*, 417(6892), pp.945–948.

Cox, R.M. & Calsbeek, R., 2009. Sexually Antagonistic Selection, Sexual Dimorphism, and the Resolution of Intralocus Sexual Conflict. *American Naturalist*, 173(2), pp.176–187.

Crespi, B. & Semeniuk, C., 2004. Parent-offspring conflict in the evolution of vertebrate reproductive mode. *Am Nat*, 163(5), pp.635–53.

Curley, J.P. et al., 2004. Coadaptation in mother and infant regulated by a paternally expressed imprinted gene. *Proceedings. Biological sciences / The Royal Society*, 271(1545), pp.1303–1309.

Dahms, N.M. et al., 1993. Characterization of mannose-6-phosphate receptors (MPRs) from opossum liver: opossum-cation independent MPR binds insiln-like growth factor-II. *Endocrinology*, 133, pp.440–446.

Dawkins, R., 1989. *The selfish gene* 6th Editio., New York: Oxford University Press.

Dawson, W.D., 1965. Fertility and Size Inheritance in a Peromyscus Species Cross. *Evolution*, 19(1), pp.44–55.

Dawson, W.D. et al., 1993. Growth regulation in peromyscus species hybrids - a test for mitochondrial nuclear genomic interaction. *Growth, Development and Aging\r57*, pp.121–134.

Day, T. & Bonduriansky, R., 2004. Intralocus Sexual Conflict Can Drive the Evolution of Genomic Imprinting. *Genetics*, 167(4), pp.1537–1546.

DeChiara, T.M., Efstratiadis, A. & Robertson, E.J., 1990. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature*, 345, pp.78–80.

DeChiara, T.M., Robertson, E.J. & Efstratiadis, A., 1991. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell*, 64(4), pp.849–859.

Doerfler, W., 1991. Patterns of DNA methylation-evolutionary vestiges of foreign DNA inactivation as a host defense mechanism. *Biological chemistry Hoppe-Seyler*, 372(8), pp.557–564.

Eversole-Cire, P. et al., 1993. Activation of an imprinted Igf 2 gene in mouse somatic cell cultures. *Molecular and cellular biology*, 13(8), pp.4928–38.

Faber, J.J., Thornburg, K.L. & Binder, N.D., 1992. Physiology of Placental Transfer in Mammals. *American Zoologist*, 32(2), pp.343–354.

Feil, R. et al., 1994. Developmental control of allelic methylation in the imprinted mouse Igf2 and H19 genes. *Development*, 120(10), pp.2933–2943.

Ferguson-Smith, A.C. et al., 1993. Parental-origin-specific epigenetic modifications of the mouse H19 gene. *Nature*, 362, pp.751–755.

Glenn, C.C. et al., 1996. Gene structure, DNA methylation, and imprinted expression of the human SNRPN gene. *American journal of human genetics*, 58(2), pp.335–46. Haig, D., 2014. Coadaptation and conflict, misconception and muddle, in the evolution of genomic imprinting. *Heredity*, 113(2), pp.96–103.

Haig, D., 2004. Genomic imprinting and kinship: how good is the evidence? *Annual review of genetics*, 38, pp.553–585.

Haig, D., 1996. Placental hormones, genomic imprinting, and maternal-fetal communication. *Journal of Evolutionary Biology*, 9(3), pp.357–380.

Haig, D., 2000. The Kinship Theory of Genomic Imprinting. *Annual Review of Ecology and Systematics*, (34), pp.9–32.

Haig, D. & Graham, C., 1991. Genomic Imprinting and the Strange Case of the Insulin-like Growth Factor II Receptor. *Cell*, 64, pp.1045–1046.

Haig, D. & Westoby, M., 1991. Genomic imprinting in endosperm - its effect on seed development in crosses between species, and between different ploidies of the same

species, and its implications for the evolution of apomixis. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 333(1266), pp.1–13.

Haig, D. & Westoby, M., 1989. Parent-Specific Gene Expression and the Triploid Endosperm. *The American Naturalist*, 134(1), p.147.

Haig, D. & Wilczek, A., 2006. Sexual conflict and the alternation of haploid and diploid generations. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 361(1466), pp.335–343.

Hark, A.T. et al., 2000. CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. *Nature*, 405(6785), pp.486–489.

Hollenberg, F. & Wourms, J.P., 1995. Embryonic growth and maternal nutrient sources in goodeid fishes (Teleostei: Cyprinodontiformes). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 271(5), pp.379–394.

Ideraabdullah, F.Y., Vigneau, S. & Bartolomei, M.S., 2008. Genomic imprinting mechanisms in mammals. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, 647(1–2), pp.77–85.

Kaneko-Ishino, T., Kohda, T. & Ishino, F., 2003. The Regulation and Biological Significance of Genomic Imprinting in Mammals. *Journal of Biochemistry*, 133(6), pp.699–711.

Keverne, E.B. & Curley, J.P., 2008. Epigenetics, brain evolution and behaviour. *Frontiers in Neuroendocrinology*, 29(3), pp.398–412.

Killian, J.K. et al., 2001. Divergent evolution in M6P/IGF2R imprinting from the Jurassic to the Quaternary. *Human Molecular Genetics*, 10(17), pp.1721–1728.

Killian, J.K. et al., 2000. M6P/IGF2R imprinting evolution in mammals. *Molecular cell*, 5(4), pp.707–716.

Killian, J.K. et al., 2001. Monotreme IGF2 expression and ancestral origin of genomic imprinting. *Journal of Experimental Zoology*, 291(2), pp.205–212.

Knowles, L.L. & Markow, T.A., 2001. Sexually antagonistic coevolution of a postmating-prezygotic reproductive character in desert Drosophila. *Proceedings of the National Academy of Sciences of the United States of America*, 98(15), pp.8692–6.

Kornfeld, S. & Mellman, I., 1989. The biogenesis of lysosomes. *Annu. Rev. Cell Biol.*, 5, pp.483–525.

Lau, M.M.H. et al., 1994. Loss of the imprinted IGF2/cation-independent mannose 6-phosphate receptor results in fetal overgrowth and perinatal lethality. *Genes and Development*, 8(24), pp.2953–2963.

Lawton, B.R. et al., 2005. Allelic expression of IGF2 in live-bearing, matrotrophic fishes. *Development Genes and Evolution*, 215, pp.207–212.

Leighton, P.A. et al., 1995. An enhancer deletion affects both H19 and Igf2 expression. *Genes and Development*, 9(17), pp.2079–2089.

Lessells, C.M. & McNamara, J.M., 2012. Sexual conflict over parental investment in repeated bouts: negotiation reduces overall care. *Proceedings of the Royal Society B: Biological Sciences*, 279(1733), pp.1506–14.

Lewin, B., 2004. *Genes VIII*, Pearson Prentice Hall.

Lin, B., 1984. Ploidy barrier to endosperm development i n maize. *Genetics Society of America*, 107, pp.103–115.

Lombardi, J. & Wourms, J.P., 1988. Embryonic growth and trophotaenial development in goodeid fishes (Teleostei: Atheriniformes). *Journal of Morphology*, 197(2), pp.193–208.

Lombardi, J. & Wourms, J.P., 1979. Structure, function, and evolution of trophotaeniae, placental analogues of viviparous fishes. *American Zoologist*, 19(3), p.976.

Lombardi, J. & Wourms, J.P., 1985a. The trophotaenial placenta of a viviparous goodeid fish. I: Ultrastructure of the Internal Ovarian Epithelium, the Maternal Component. *Journal of molecular biology*, 184, pp.277–292.

Lombardi, J. & Wourms, J.P., 1985b. The trophotaenial placenta of viviparous goodeid fish. II. Ultrastructure of the trophotaenia, the embryonic component. *Journal of Morphology*, 184(3), pp.293–309.

Ludwig, T. et al., 1996. Mouse Mutants Lacking the Type 2 IGF Receptor (IGF2R) Are Rescued from Perinatal Lethality …. *Developmental Biology*, 535(182), pp.517–535.

Macías-Garcia, C. & González-Zuarth, C.A., 2005. Reproductive Behavior of Viviparous Fish and Intersexual Conflict. In M. C. Uribe & H. J. Grier, eds. *Viviparous fishes*. New Life Publication, pp. 271–284.

Mertineit, C. et al., 1998. Sex-specific exons control DNA methyltransferase in mammalian germ cells. *Development (Cambridge, England)*, 125, pp.889–897.

Moore, T. et al., 1997. Multiple imprinted sense and antisense transcripts, differential methylation and tandem repeats in a putative imprinting control region upstream of mouse Igf2. *Proceedings of the National Academy of Sciences of the United States of America*, 94(23), pp.12509–12514.

Moore, T. & Haig, D., 1991. Genomic imprinting in mammalian development: a parental tug-of-war. *Trends in Genetics*, 7(2), pp.45–49.

Morgan, D.O. et al., 1987. Insulin-like growth factor II receptor as a multifunctional binding protein. *Nature*, 329(6137), pp.301–7.

Morison, I.M. & Reeve, A.E., 1998. Insulin-like growth factor 2 and overgrowth: Molecular biology and clinical implications. *Molecular Medicine Today*, 4(3), pp.110–115.

Mossman, H.W., 1991. Comparative Morphogenesis of the Fetal Membranes Accesso and Uterine Structures. *Placenta*, 12(1), pp.1–5.

Murrell, A., Heeson, S. & Reik, W., 2004. Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. *Nature genetics*, 36(8), pp.889–893.

Nolan, C.M. et al., 2001. Imprint status of M6P/IGF2R and IGF2 in chickens. *Development Genes and Evolution*, 211(4), pp.179–183.

O'Neill, M.J. et al., 2000. Allelic expression of IGF2 in marsupials and birds. *Development genes and evolution*, 210, pp.18–20.

Okada, K. et al., 2014. Sexual conflict over mating in Gnatocerus cornutus ? Females prefer lovers not fighters. *Proceedings. Biological sciences / The Royal Society*, 281(May), p.20140281.

Osorno, J.L. & Székely, T., 2004. Sexual conflict and parental care in magnificent frigatebirds: Full compensation by deserted females. *Animal Behaviour*, 68(2), pp.337–342.

Parker, G.A., 2006. Sexual conflict over mating and fertilization: an overview. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 361(1466),

pp.235–259.

Patzner, R.A., 2008. Reprodictive strategies of fish. In M. Joao Rocha, A. Arukwe, & B. G. Kapoor, eds. *Fish reproduction. 311-250. (eds). Science Publishers, USA.* Science Publisher, pp. 211–250.

Poirier, F. et al., 1991. The murine H19 gene is activated during embryonic stem cell differentiation in vitro and at the time of implantation in the developing embryo. *Development*, 113, pp.1105–1114.

Renfree, M.B. et al., 2009. Evolution of genomic imprinting: insights from marsupials and monotremes. *Annual review of genomics and human genetics*, 10, pp.241–262.

Renfree, M.B., 1982. Implantation and placentation. In C. R. Austin & R. V. Short, eds. *Reproduction in mammals pp. 26-69, 2*. Cambridge University Press, pp. 26–69.

Renfree, M.B. et al., 2012. The origin and evolution of genomic imprinting and viviparity in mammals The origin and evolution of genomic imprinting and viviparity in mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, p.20120151.

Reznick, D.N., Mateos, M. & Springer, M.S., 2002. Independent Origins and Rapid Fish Genus Poeciliopsis. *Science*, 298, pp.1018–1020.

Rice, W.R., 1998. Intergenomic conflict, interlocus antagonistic coevolution, and the evolution of reproductive isolation. In *Endless forms: species and speciation*. New York: Oxford University Press, pp. 261–270.

Rice, W.R. & Holland, B., 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behav Ecol and Sociobiol*, 41, pp.1–10.

Rogers, J.F. & Dawson, W.D., 1970. Foetal and placental size in a Peromyscus species cross. *J Reprod Fertil*, 21(2), pp.255–262.

Saldivar Lemus, Y. et al., 2017. Asymmetric paternal effect on offspring size linked to parent-of-origin expression of an insulin-like growth factor. *Ecology and Evolution*, (April), pp.1–48.

Sandhu, K.S., 2010. Systems properties of proteins encoded by imprinted genes. *Epigenetics*, 5(7), pp.627–636.

Sara, V.R. & Hall, K., 1990. Insulin-like Growth Factors and their Binding Proteins. *Physiological Reviews*, 70(3), pp.591–614.

Sato, M. & Stryker, M.P., 2010. Genomic imprinting of experience-dependent cortical plasticity by the ubiquitin ligase gene Ube3a. *Proceedings of the National Academy of Sciences*, 107(12), pp.5611–5616.

Schrader, M. & Travis, J., 2008. Testing the viviparity-driven-conflict hypothesis: parent-offspring conflict and the evolution of reproductive isolation in a poeciliid fish. *The American naturalist*, 172(6), pp.806–817.

Scott, R.J. et al., 1998. Parent-of-origin effects on seed development in Arabidopsis thaliana. *Development*, 125(17), pp.3329–3341.

Tilghman, S.M., 1999. The sins of the fathers and mothers: Genomic imprinting in mammalian development. *Cell*, 96(2), pp.185–193.

Tremblay, K.D. et al., 1995. A paternal-specific methylation imprint marks the alleles of the mouse H19 gene. *Nature Genetics*, 9, pp.407–413.

Tremblay, K.D., Duran, K.L. & Bartolomei, M.S., 1997. A 5' 2-kilobase-pair region of the imprinted mouse H19 gene exhibits exclusive paternal methylation throughout development. *Molecular and cellular biology*, 17(8), pp.4322–4329.

Trivers, R.L., 1974. Parent-Offspring Conflict. *American Zoologist*, 14(1), pp.249–264.

Verona, R.I., Mann, M.R.W. & Bartolomei, M.S., 2003. GENOMICI MPRINTING: Intricacies of Epigenetic Regulation in Clusters. *Annual Review of Cell and Developmental Biology*, 19(1), pp.237–259.

Vrana, P.B., 2007. Genomic Imprinting As a Mechanism of Reproductive Isolation in Mammals. *Journal of Mammalogy*, 88(1), pp.5–23.

Wilkins, J.F. & Haig, D., 2001. Genomic imprinting of two antagonistic loci. *Proceedings. Biological sciences / The Royal Society*, 268(1479), pp.1861–7.

Wilkins, J.F. & Haig, D., 2003. What good is genomic imprinting: the function of parent-specific gene expression. *Nature Reviews Genetics*, 4(5), pp.359–368.

Wolf, J.B., 2013. Evolution of genomic imprinting as a coordinator of coadapted gene expression. *Proceedings of the National Academy of Sciences*, 110(13), pp.5085–5090.

Wolf, J.B. & Brodie III, E.D., 2009. The Coadaptation of Parental and Offspring Characters. *Evolution*, 52(2), pp.299–308.

Wolf, J.B. & Hager, R., 2006. A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLoS Biology*, 4(12), pp.2238–2243.

Wolf, J.B., Oakey, R.J. & Feil, R., 2014. Imprinted gene expression in hybrids: perturbed mechanisms and evolutionary implications. *Heredity*, 113(2), pp.167–75.

Wourms, J.P., 1981. Viviparity: The maternal-fetal relationship in fishes. *Integrative and Comparative Biology*, 21(2), pp.473–515.

Wourms, J.P., Grove, B. & Lombardi, J., 1988. The maternal-embryonic relationship in viviparous fishes. In W. Hoar & D. Randall, eds. *Fish Physiology*. New York: Academic Press, pp. 1–134.

Wutz, A. & Barlow, D.P., 1998. Imprinting of the mouse Igf 2 r gene depends on an intronic CpG island. , 140, pp.9–14.

Xie, B. et al., 2009. The evolutionary foundation of genomic imprinting in lower vertebrates. *Chinese Science Bulletin*, 54(8), pp.1354–1360.

Yandell, C.A. et al., 1999. PROTEIN CHEMISTRY AND STRUCTURE : The Kangaroo Cation-independent Mannose 6-Phosphate Receptor Binds Insulin-like Growth Factor II with Low Affinity The Kangaroo Cation-independent Mannose 6-Phosphate Receptor Binds Insulin-like Growth Factor II with Low . , 274(38), pp.27076–27082.

Yoder, J.A. et al., 1997. DNA (cytosine-5)-methyltransferases in mouse cells and tissues. Studies with a mechanism-based probe. *Journal of molecular biology*, 270(3), pp.385–395.

Yuan, X.-N. et al., 2011. Functional conservation and divergence of duplicated insulin-like growth factor 2 genes in grass carp (Ctenopharyngodon idellus). *Gene*, 470(2011), pp.46–52.

Zeh, D.W. & Zeh, J. a, 2000. Reproductive mode and speciation: the viviparity-driven conflict hypothesis. *BioEssays*, 22(10), pp.938–46.

Figure 1. Scheme of the evolution of genomic imprinting among vertebrates. Genomic imprinting has been documented in Therian mammals, and it is absent in monotremes and birds. There have been few attempts to find genomic imprinting in reptiles, amphibians and fish; therefore it is hard to determine if these groups have evolved parent-of-origin gene expression.

Figure 2. Expression pattern of *igf2* in mouse embryos. The paternal allele of *igf2* is active, while *H19* allele is silent. The primary DMR that is located in the intergenic zone between *igf2* and *h19* is hypermethylated, which impedes the binging of CTCF proteins to the DNA so that enhancers can act on the promoter of *igf2.* Additionally, a secondary DMR forms a loop that promotes contact between the *igf2* promoter and the enhancers. Meanwhile, since this primary DMR includes a section of the promoter of *H19,* the enhancers cannot act on it and *h19* remains silent. In contrast the maternal allele of *igf2* remains silent while h19 is transcribed. The primary DMR is hypomethylated in the maternal allele. This allows the CTCF proteins to bind and block the enhancers' activity on *igf2* promoter. The *H19* promoter is maternally hypomethylated and therefore the gene is transcribed.

Figure 3. Stages of genomic imprinting during development: i) Erasure: the imprint (inherited from the previous generation -generation one-) is erased on both parental chromosomes during germ cell development. ii) Establishment: according to the sex of the germ line a new imprint is established (+ or -) for the next generation (generation two); iii) Maintenance: the imprint is stably transmitted during mitosis; iiii) Reading: the maternal and paternal imprints are translated into monoallelic expression during development. (Modified from Constância et al. 1998).

Figure 4. Phylogeny of vertebrates. Grey stars indicate the taxa where viviparity (including matrotrophy and lecithotrophy) has evolved.

Figure 1.

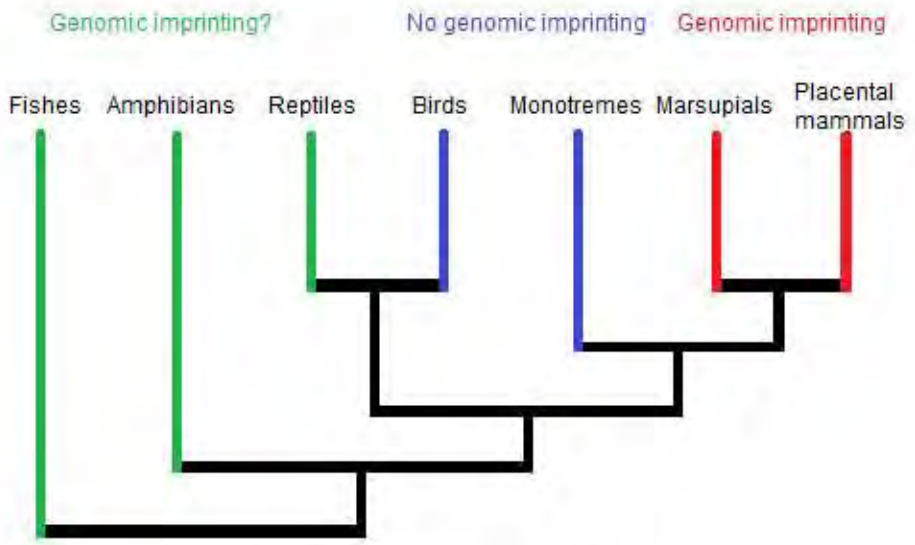

Genomic imprinting?
No genomic imprinting
Genomic imprinting
Fishes
Amphibians
Reptiles
Birds
Monotremes
Marsupials
Placental mammals

Figure 2.

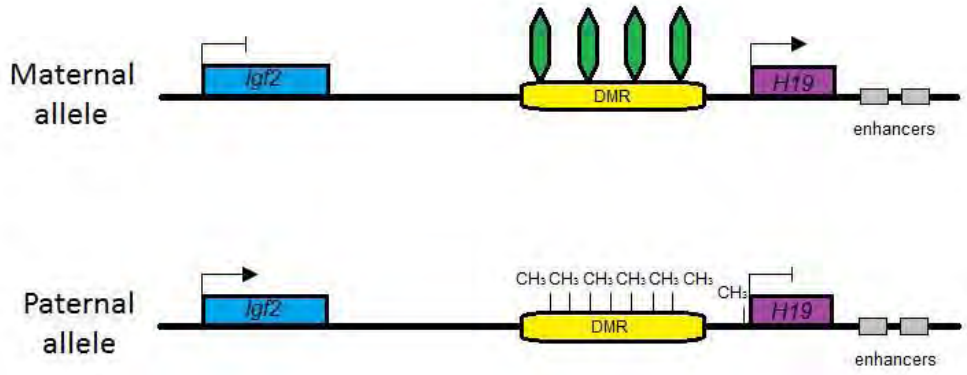

Maternal allele
Igf2
DMR
H19
enhancers
Paternal allele
CH3 CH3 CH3 CH3 CH3 CH3
CH3
Igf2
DMR
H19
enhancers

Figure 3.

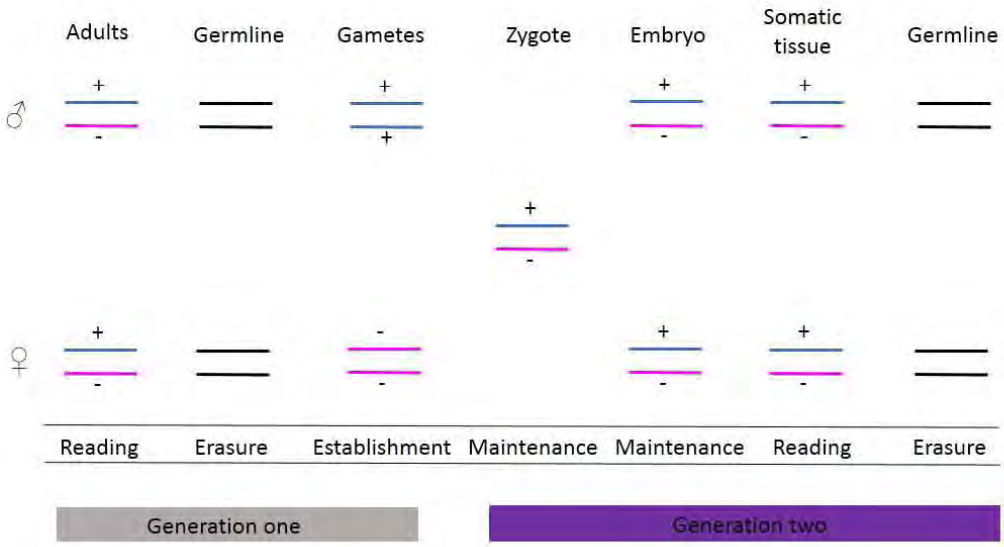
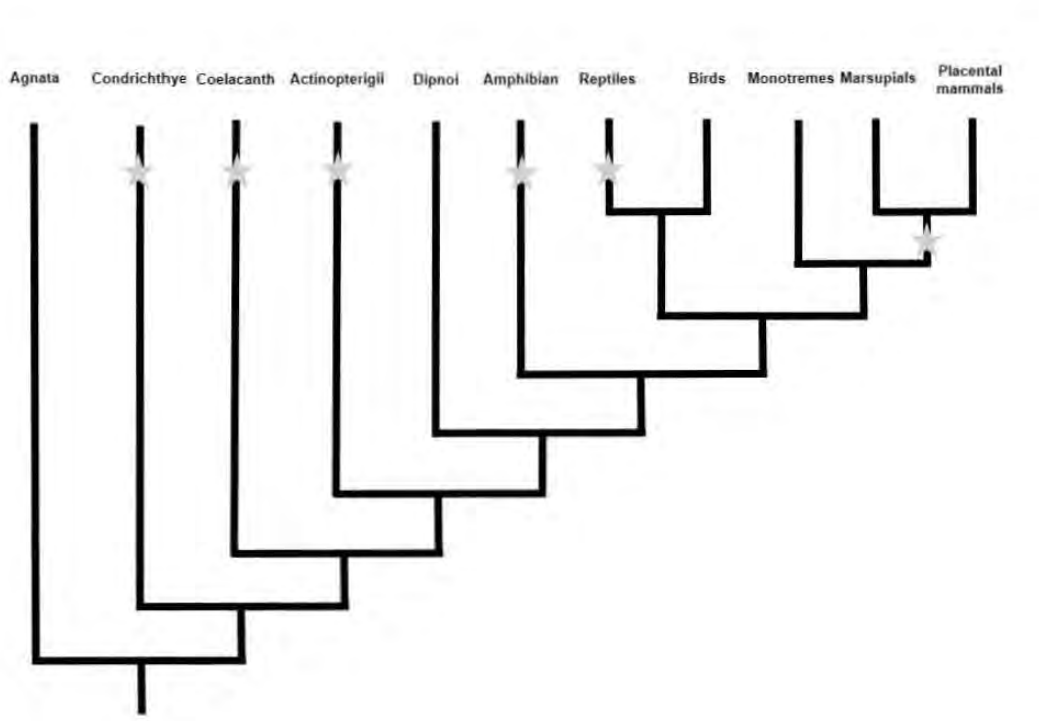

Adults
Germline
Gametes
Zygote
Embryo
Somatic tissue
Germline
♂
♀
Reading
Erasure
Establishment
Maintenance
Maintenance
Reading
Erasure
Generation one
Generation two

Figure 4.


Agnata
Condrichthye
Coelacanth
Actinopterigii
Dipnoi
Amphibian
Reptiles
Birds
Monotremes
Marsupials
Placental mammals

CHAPTER 4

The evolution of viviparity, matrotrophy and placentrotrophy set the arena for sexual conflict over the optimal maternal allocation of resources (for each sex) during embryonic development, where male adaptations to influence maternal investment have evolved. This conflict has been demonstrated in viviparous mammals, but there are some other groups of vertebrates, such as matrotrophic and placental fish, where this conflict could have evolved.

¿Efectos asiméticros paternos en la talla de los críos revelan manipulación de la inversión materna?

Do asymmetric paternal effects on offspring size reveal male manipulation of maternal investment?

Yolitzi Saldivar Lemus[1], Jean-Philippe Vielle-Calzada[2], Michael G. Ritchie[3] & Constantino Macías Garcia[1]

[1]Instituto de Ecología, Universidad Nacional Autónoma de México, [2] UGA Laboratorio Nacional de Genómica para la Biodiversidad CINVESTAV Irapuato México, [3]School of Biology, University of St. Andrews.

Manuscript

This manuscript is a more detailed version of the phenotypic data publised in the article of Appendix 5

Abstract. Sexual conflict can lead to manipulation of reproductive investment of interacting partners. Males that can manipulate maternal investment in their progeny would benefit if this leads to more successful offspring than those of their competitors, even if it reduces female potential future reproduction investment. Where embryos can influence maternal investment, paternal genetic manipulation may evolve, prompting the evolution of maternal countermeasures. A way of detecting such coevolution is to examine crosses between populations, as asymmetric embryonic growth may occur upon secondary contact if the coevolution is no longer matched. Appropriate models include matrotrophic fish, where maternal investment is an important component of life history. We sought evidence of asymmetry in reciprocal crosses between two genetically distant populations of the Amarillo fish (*Girardinichthys multiradiatus*): Zempoala (Z) and San Matías (M). Whereas offspring from M females and Z males were indistinguishable from their controls (M-M), offspring from Z females and M males were significantly bigger and heavier than their controls (Z-Z). Hybrid vigor cannot explain this difference, since M-Z offspring were smaller than those of Z-M. We also rule out the possibility that Z females found M males more attractive and thus preferentially invested on their offspring because females from both populations prefer sympatric males. Our data, although consistent with the breakdown of a coadaptation between mother and offspring, are more consistent with expectations if antagonistic coevolution in M promoted enhanced male manipulation of female reproductive allocation coupled with enhanced female resistance, in comparison with Z, where females seem to be unable to resist such manipulation. This preliminary conclusion should be assessed by evaluating the pattern of expression of genes responsible for maternal-embryonic communication.

Key words: sexual conflict, antagonistic coevolution, parental investment, matrotrophy, viviparous fish, Goodeidae.

Introduction

Whenever members of different sexes interact, there is potential for sexual conflict to occur since the evolutionary interests of both sexes in relation to the outcome of the interaction are normally different (Parker 1979; Parker 2006). Conflict can arise in relation to current or future mating decisions (Parker 1974; Smuts & Smuts 1993; Magurran & Seghers 1994; Arnqvist & Rowe 2002), and also in relation to how much each individual should invest in progeny (Trivers 1972). Thus, members of a breeding pair may desert parental care (e.g. Osorno & Székely 2004; see Maynard Smith 1977), leaving the partner to do all the remaining of the investment (McNamara et al. 2003), they may negotiate the amount of care each provide (based, for instance, on the condition of the offspring) or manipulate the partner into performing more investment (Lessells & McNamara 2012).

Even if offspring are cared exclusively by members of one sex (usually by females), manipulation may still be adaptive if females may be induced to invest preferentially in the brood of the current male, either through sensory manipulation (e.g. Burley 1986; Gil et al. 1999; Velando et al. 2006), or by enhancing the ability of the offspring to extract resources from the mother. In viviparous species, this has been achieved by the overexpression of genes in the offspring that regulate the maternal-foetal feeding communication, leading to the evolution of a particular pattern of allelic expression known as genomic imprinting. Indeed, the explanation for the evolution of genomic imprinting is the conflict of interests between mother and offspring, or between the paternal and maternal alleles in the offspring (Haig & Westoby 1989; see also Tilghman 1999; Haig 2000; Constância et al. 2002)

Viviparity implies a close physiological association between embryos and mother, which promotes offspring survival because of regular direct provisioning and protection (Blackburn 1999), while allowing females to adaptively adjust the amount and rate of resource delivery (Trexler & DeAngelis 2003). Matrotrophic females must trade those benefits against the increased vulnerability incurred when pregnant as they become bulky and encumbered, and

the difficulty of producing large broods repeatedly (Seigel et al. 1987; Miles et al. 2000; Bleu et al. 2012). Additionally, since reproduction is energetically very costly for females, they must trade-off between current reproduction and survival, future reproduction and growth (Stearns 1992). Yet the balance between those trade-offs is different for males, as they do not pay the costs but would enjoy greater benefits if the females they mate with increase their investment and produce bigger offspring or larger broods than they would otherwise (Crespi & Semeniuk 2004; Griggio et al. 2009). Hence species with more maternal investment may be more prone to antagonistic selection on investment in any current breeding episode.

Antagonistic manipulation can lead to resolution of conflict if males' and females' adaptations are constatly coevolving, and thus the effects of such manipulation are difficult to detect (Arnqvist & Rowe 2005); but one powerful method of finding evidence of antagonism is to cross populations, which may occupy different positions in cycles of antagonistic coevolution. Sexual conflict can lead to evolutionary divergence (Chapman et al. 2003; Arnqvist & Rowe 2005), as adaptations that are beneficial for the members of one sex prompt the evolution of countermeasures on the other to mitigate their negative effects (Arnqvist & Rowe 2002). In the case of viviparous species, excessive male-induced increases of offspring demands may reduce the mother's lifetime breeding success, thus females that develop effective means to resist this manipulation would be selected, leading to a coevolutionary arms-race (a form of Intergenomic Contest Evolution, or ICE; Rice & Holland 1997). It is likely that independent populations or species will differ in details of such coevolution (Parker & Partridge 1998; Rowe et al. 2003).

For example, when a female of monogamous *Peromyscus polionotus* mated with males of a polygynous *P. maniculatus*, neonates were considerably larger than any of the progenitors and placentas were 5-6 times greater in mass than those of the opposite cross (Rogers & Dawson 1970). Further examples have been found in plants (reviewed in Alleman & Doctor

2000), and insects, where fertility of honeybees has been shown to be influenced by epigamic male manipulation (Oldroyd et al. 2014). Additionally, Schrader and Travis (2008; 2009) conducted inter-population crosses of the placental matrotrophic fish *Heterandria formosa,* and found that 1) when a female of a relatively monandrous population mates with male from a relatively polyandrous population, the ratio of spontaneous abortion is higher than in the opposite cross, and 2) that embryos can influence maternal investment and that investment is traded versus fecundity.

Viviparity has been considered the main driver of population divergence because of the close and particular physiological interactions between mother and embryos. This may lead to a conflict between them or between both parents over the level of maternal investment (Zeh & Zeh 2000; Trivers 1974). Matrotrophy, an advanced form of viviparity involving maternal provisioning of embryos through gestation, is present in many groups of vertebrates (Blackburn 2015), and among fishes in at least 11 families, where it may have evolved independently (Wourms et al. 1988). Within the Actinopterygii, matrotrophy is remarkable in some species of Poeciliidae, e.g. in the placental *Heterandria formosa*, and in the Mexican Goodeidae (Goodeinae; Lombardi & Wourms 1985a; Lombardi & Wourms 1985b), another viviparous group with advanced matrotrophy. This is a clade of ca. 40 species distributed in 17 or 18 genera (Webb et al. 2004), a ratio of genera to species that may be due to rapid speciation led by geographic isolation and either by the evolution of viviparity, or by the high prevalence of sexual selection, itself linked to the extreme sexual asymmetry in parental care that viviparity entails (Smith 1980; Ritchie et al. 2005; Macías Garcia 2014). Such asymmetry in parental investment is particularly large in the Goodeinae; in which females nourish their embryos through unique specialized embryonic tissues known as trophotaenia (Schindler 2005; Uribe & García Alarcón 2005), for 7-8 weeks (Macías-Garcia & Saborío 2004), during which they grow up to 15 000% (Lombardi & Wourms 1985a). Extended maternal provisioning and a specialized placenta-like structure

makes Goodeinae fish potentially good models for the study of antagonistic coevolution of parental allocation of resources to developing embryos, but no previous study has examined this. We evaluated this possibility using crosses between two genetically distant populations of the Amarillo (*Girardinichthys multiradiatus*), a goodeine from Central Mexico.

Girardinichthys multiradiatus, the Amarillo, is found in water bodies of the upper Lerma River basin, and in limited upland regions of the adjacent Balsas and Pánuco catchments (Gesundheit & Macias-Garcia 2005). Males have much larger and colourful median fins than the females, who base their mate choice on those attributes and on courtship performance (Macias-Garcia et al. 1994; Macías Garcia et al. 1998; González Zuarth & Macías Garcia 2006). There has been rapid population divergence (Ritchie et al. 2007; Macías Garcia et al. 2012), and female mate choice often –but not always- leads to pre-mating isolation between populations (González Zuarth & Macías Garcia 2006; Macías Garcia et al. 2012).

For this study we selected the two populations that are most distant geographically and genetically; Zempoala (Z), a mountain population in the watershed between the southernmost reaches of the Lerma and the Balsas catchments, and San Matías (M), in the Balsas basin, at the north-western corner of the Amarillo distribution (Macías Garcia et al. 2012).

The main aim of this manuscript is to determine if males of *Girardinichthys multiradiatus* can manipulate (increase) the maternal allocation of resources during embryonic development, and if such manipulation has lead the evolution of an antagonistic coevolution between males and females. If this is true, since Z and M populations have been evolving in isolation and therefore they should be in a different point of such coevolution, we predict we will find asymmetric effects on phenotypic traits of offspring from inter-population crosses compared to those from the intra-population crosses.

Methods

Adult fish were collected from the field (SAGARPA permit DGOPA/01262/040310.0716) using hand nets, and promptly transported to aquaria at the Instituto de Ecología, UNAM in plastic bags containing local water, Stress Coat™ and antiseptics. To avoid possible effects due to differences in the environment experienced by fish from the two localities during development we obtained and raised the first generation (F1) in captivity under standardized conditions (12-hour-day-night cycle, 21ºC and fed SeraVipan™ commercial fish flakes twice a day). Newborns were kept in 80-L population-specific aquaria until sex could be determined at between 60 and 125 days of age (100 ± 16 days; see De Gasperin & Macías Garcia 2014), when each fish was assigned to one of the following crosses (female-male): 1) M-M, 2) Z-Z, 3) M-Z, and 4) Z-M.

Females were kept with the appropriate males either in one communal 80-L tank per cross (n = 49 females, or 62% of the final sample) or in smaller groups of one or two pairs, but at a comparable density within 20-L tanks (n = 30 females, 38% of the final sample) until females showed signs of early pregnancy, when they were gently moved to individual 20-L maternity tanks and weighed initially once a week, and every two days as birth became imminent (usually in weeks 7 and 8). The distribution of females kept in either condition was similar for all crosses ($x^2 = 1.2$, $df = 3$, $p = 0.75$). For weighing, a dish containing water from the fish aquarium and Stress Coat™ was placed on top of an electronic scale, then we set the tare and the female was introduced in the dish after carefully moping the excess of water from her body. Females were also weighed immediately after parturition, and their body length (standard length) and width were measured from digital photographs taken the following day (to ensure that all offspring had been delivered) on a narrow chamber against a millimeter paper using UTHSCSA ImageTool freeware.

Individual offspring were measured in the same way as their mothers, but their mass was obtained by weighing the whole brood, then dividing the value by the number of fish, to

reduce error and minimize potentially harmful manipulation. Other variables collected were brood size (all fish born, whether alive or dead), offspring survival (number of offspring alive at the end of the 4th day after birth / brood size), and reproductive allocation (RA; brood weight / female weight after parturition + brood weight).

We did not measure male length, since females and males were of the same cohort, then we assumed they were of the relatively same size.

Some females died after or during giving birth, and before all her measures were taken, therefore the sample size of female variables differed. We included in the analysis the information of their broods because that data was complete. Discrepancies in sample sizes of female size in M-M cross and offspring size in Z-Z cross were due to female mortality after birth but before all her measures were taken, and to the loss of the photos of the brood. Eight broods were composed of dead fish that afforded no reliable estimates of offspring size and/or mass, but they strengthened the information about brood size and offspring survival. Samples sizes among treatments were very variable mainly because of breeding success.

To control for female size, which varied considerably (Table 1) and can influence brood- (Macías-Garcia & Saborío 2004) and offspring size, we entered female length (SL) as a co-variable in the analyses (female SL was highly and significantly correlated with female width; $r = 0.93$, $F_{(1,70)} = 418.7$, $p < 0.0001$). We compared breeding performance and brood attributes using mixed models (GLM) in which each brood was entered only once in each analysis (brood size, reproductive allocation [RA]-reported as the ratio of reproductive biomass/total biomass (Abrahamson & Gadgil 1973) -, offspring survival, mean offspring mass and the proportion of over-sized embryos by brood), and when individual data were available for each newborn (individual offspring SL and width) data were nested within brood. All our mixed models included female SL, and raising environment as covariates, and female population of origin as one fixed factor; they also included male population of origin

and the interaction between male and female population, since these two effects would be indicative of offspring genotype influencing female parental investment (Reznick 1981; Reznick 1982; see Schrader & Travis 2009). Reported post-hoc probabilities are corrected (Bonferroni) for multiple comparisons. All analyses were performed using NCSS 2007 v. 7.1.21.

Results

As we have noticed when collecting fish from these localities (CMG unpublished data) F1 females from Z were smaller than those from M ($t = 4.57$, $df = 70$, $p < 0.0001$; Table 1), but did not produce smaller offspring than M females when mated with males of their own population (Bonferroni $F_{(1,67.1)}=0.93$, $p=1.0$). This inversion was enhanced when females from Z mated with M males, as they had bigger offspring than when they mated with Z males (Bonferroni $F_{(1,63.0)}=9.83$, $p=0.02$). Also, we detected a significant interaction between male and female population of origin ($F_{(1,64.4)} = 7.45$, $p = 0.008$) and a significant effect of the cross ($F_{(3,64.6)} = 3.7$, $p = 0.02$). We observed a similar pattern with offspring width, with no effect of female population ($F_{(1,65.2)} = 0.94$, $p = 0.34$), and a significant male X female interaction ($F_{(1,65.2)} = 4.51$, $p = 0.04$); offspring of Z-M were wider than those from Z-Z crosses (Bonferroni $F_{(1,63.5)} = 7.14$, $p = 0.02$; Figure 1b). We note that neither length, nor width of offspring from M females differ between crosses (length, Bonferroni $F_{(1,65.6)} = 1.17$, $p = 0.57$; width, Bonferroni $F_{(1,66.4)} = 0.32$, $p = 1$; see appendix Tables S1 and S2).

Neonates produced by M females were heavier than those produced by the smaller Z females ($F_{(1,66.0)} = 5.15$, $p = 0.03$; Table 1; Figure 1c). Weight significantly co-varied with maternal length ($F_{(1,66.0)} = 6.21$, $p = 0.015$). As above, Z females mated with M males produced heavier offspring than their controls ($F_{(1,66.0)} = 7.17$, $p = 0.02$), although the male X female interaction was not significant.

Offspring size mainly ranged between 25 and 40% the size of the mother, however, some females gave birth to a few offspring whose size varied between 40 and 50% their own size. Offspring size may vary within broods as an adaptive result of unpredictable environments (Marshall et al. 2008); however, embryophagy, the intraovaric consumption of siblings (Greven & Grossherr 1992), seems to produce a similar effect. In Goodeids some embryos absorb gradually their siblings by surrounding them with their trophotanie, and reach larger sizes than their own sibling (pers. Observations).

The proportion of those over-sized offspring was normally above 15% in intrapopulation crosses; nevertheless, females from Z produced more over-sized offspring than M females (Bonferroni $F_{(1,67)}$=6.82, p = 0.01), and M males could significantly enhance this effect when mated with Z females than when mated with M females (Bonferroni $F_{(1,67)}$=6.82, p = 0.02, see Appendix 1 Figure S1). This size increase in offspring from Z-M cross is unlikely to be related to an increment in embryophagy in Z-M broods since the brood size was not reduced in Z-M cross compared to Z-Z cross (Bonferroni $F_{(1,66)} = 0.15$, $p = 1.0$; Figure 3c).

Brood size significantly covaried with female SL ($F_{(1,66.0)} = 24.14$, $p < 0.0001$), and there was also an effect of female's population of origin on brood size ($F_{(1,66.0)} = 6.30$, p= 0.01). Females from Zempoala gave birth to larger broods compared to M females (Figure 2b). Neither the parental population, nor the interaction, had a significant effect (see supplementary Table S3).

The above effects of the interaction between paternal and maternal population on offspring size were not due to differences in female reproductive allocation, which did not differ between populations, although fell short off significance ($F_{(1,532.0)} = 2.23.19$, p = 0.0814; Figure 2a) but was related to female SL ($F_{(1,523)} = 9.256.04$, p = 0.0032). In addition, although being marginally significant, M males enhanced Z reproductive allocation when comparing to M females (Bonferroni $F_{(1,52)} = 4.59$, p = 0.07).

Discussion

As we predicted, we found an interaction between the paternal and maternal genotypes in the size attained at birth by *G. multiradiatus* offspring. This is not the consequence of population differences in female size, since this was entered as a co-variable in all the analyses, nor of differences in reproductive allocation, as females from both populations invested similarly in their broods. This statistical effect is a signature of genomic conflict (Schrader & Travis 2009) and should justify the investigation of the mechanisms by which paternal and maternal genomes interact in the developing Goodeid embryos.

We also detected differences in how resources transferred to their embryos are used in both populations. While the size of their offspring was similar, the (smaller) females from Zempoala produced more, but lighter newborn fish than their San Matías counterparts. These global patterns break down when the effect of the sire is taken into account; for Zempoala females, being mated with a male from San Matías results in larger, wider and heavier offspring than if mating with a Zempoala male (Figure 1), which does not happens in the M-Z cross compared to M-M cross, further suggesting that the male genotype influences female investment in offspring in this matrotrophic fish through an interaction between the maternal and paternal contributions, where the male effect is dependent on the maternal background.

The size of offspring from matings between populations can depart from that of intrapopulation crosses in several ways. In positive heterosis (hybrid vigor, or simply "heterosis"; Shull 1908), offspring from both reciprocal crosses would be expected to be similarly larger (or healthier, or fitter) than offspring from the parental populations/strains (e.g. Shikano et al. 2000), whereas outbreeding depression would cause smaller/less fit offspring in both inter-population crosses. These two effects are expected to be symmetrical, due to either the attenuation of the mutational load (Keller & Waller 2002) or to the

breakdown of co-adapted genomes (Templeton et al. 1986), and are thus unlikely explanations for the observed phenotypic results. Furthermore, disruption of coadapted complexes in F1 hybrids are usually only seen in one sex (Haldane 1922), and although we do not have data on the sex of the newborn, we found no male X female effect in the coefficient of variation of offspring size ($F_{(1,66)} = 0.21$, $p = 0.65$), as would have been expected if Haldane's rule was at work. Co-dominance is also unlikely, since this should result in offspring from the two reciprocal crosses having the same phenotype.

Differences between parental and hybrid phenotypes can also be the consequence of maternal effects if, for instance, females perceive the males from the alternative populations to be more attractive than those from their own, and preferentially invest in offspring of attractive males (Burley 1988; Gil et al. 1999). This is unlikely to explain our results since females from both localities are reluctant to mate with males from the other population (González Zuarth & Macías Garcia 2006), unless they are raised together from an early age (e.g. as soon as their sex can be ascertained; (De Gasperin & Macías Garcia 2014). Maternal effects are also an unlikely explanation because only the offspring of M-Z, crosses, and not those from Z-M, were larger than their controls (Figure 1). Such an asymmetric effect might be generated by male-independent maternal determination of offspring size, but only in hybrid-parental backcrosses (e.g. Reznick 1981).

The disruption of gene coadaptation resulted from interpropulation crosses may also lead to this type of phenotypic results. Such coadaptations can be among imprinted genes within a cluster (Wolf 2013), or between the maternal and embryonic genotypes (Wolf & Brodie III 2009), and its breakdown in hybrid offspring may result in gene silencing (Ortíz-Barrientos et al. 2007) or in the disruption of genomic imprinting (Wolf et al. 2014).

Our results are also, and maybe even more consistent, with the expectations derived from the theory of sexual conflict (Parker 1979; Parker 2006). Goodeid matrotrophic viviparity involves a massive, protracted transfer of nutrients to the embryos (Lombardi &

Wourms 1985a; Lombardi & Wourms 1985b), that seems to be co-opted by males by the influencing the maternal-embryonic communication that controls the nutrient delivery (Constância et al. 2002). Since the offspring number of Z-M and M-Z crosses was not affected, it is unlikely that the bigger size and heavier weight of the Z-M embryos was due to an increment in embryophagy. Furthermore, attributes of goodeids such as a long gestation period (8 weeks, as long as the one of some small mammals such as dogs), the trophotaenial placenta, which allows an efficient nutrient transport that is reflected in the embryo through an increase on dry weight of up to 15 000% (Lombardi & Wourms 1988), and a expensive courting behavior by males impose assymetric costs to both sexes, which may favour the evolution of conflict via male manipulation.

We do not have any evidence either suggesting that males can influence female investment through sensorial stimulation during courtship (see above), but a known mechanism enabling this control of the mother-embryo communication is by promoting the over-expression in the embryos of the proteins in charge of such communication, i.e. the insulin-like growth factors, subsequently may lead to the evolution of genomic imprinting (Moore & Haig 1991). If genomic imprinting has evolve as a result of male manipulation of maternal effort in the Amarillo, it may, as in mammals, involve the insulin-like growth factor IGF2 (DeChiara et al. 1990; DeChiara et al. 1991),a protein that plays an important role in embryonic development, promoting both growth and cellular differentiation, and that is involved in the process of nutrient exchange between mother and embryo (Constância et al. 2002). The breeding system of the Amarillo fits with the conditions stipulated by Wilkins & Haig (2003) as potential promoters of genomic imprinting; 1) females have offspring with more than one male (Macías-Garcia & Saborío 2004), 2) They bear the bulk of the reproductive costs (e.g.Lombardi & Wourms 1985a; Lombardi & Wourms 1985b), and their allocation of resources can be influenced by genes that are expressed in the embryos (e.g. *igf*2; see O'Neill et al. 2007, Y. Saldívar Lemus et al. unpublished data). A possible

mechanism of male epigenetic manipulation of maternal investment is the overexpression of *igf2* (Insulin-like growth factor 2), and now we know that this gene is imprinted in placental mammals and in marsupials, but not in monotremes or birds (O'Neill et al. 2000; see Wilkins & Haig 2003), thus research should be devoted to evaluate the possibility that genomic imprinting or some differential allelic expression of genes in charge of maternal-embryonic communication could have evolved divergently in these two populations of *G. multiradiatus* and could be responsible for the phenotypic results we obtained.

Our data indicate that *G. multiradiatus* females from different populations produce offspring of different size, but do not modify the number of offspring per brood, when mated with allopatric males. This could happen if population-specific maternal factors were playing different roles, and it is also consistent with male manipulation of female reproductive allotment; however, experimental manipulation is required to tease these possibilities apart.

While we found a significant paternal X maternal interaction due to the increase in the size of embryos in the Z-M cross, we did not find a significantly substantial decrease in embryo size in M-Z broods, which would provide evidence of co-evolved female resistance to any manipulation by the males (see Moore & Haig 1991). One possibility is that females have a rather fixed amount of resources to invest and a limit on ova to fertilize in each brood (see Figure 3). Accordingly, females from San Matías always had smaller broods than females from Zempoala, and since they did not reduce their reproductive allotment when mated with Z males, they would have provided enough resources even if the demand of nutrients by their Z-sired embryos was low (Figures 2a). We did not measure how giving birth to bigger offspring affected negatively females' fitness, i.e. by diminishing their longevity, growth or their future brood and offspring size.

Our data suggest that 1) males of *G. multiradiatus* can manipulate female reproductive investment and 2) that females have evolved countermeasures to resist such manipulation (antagonistic coevolution). Females from different populations invest

differently in offspring size, but not in brood size, when mated with allopatric males. This effect, while asymmetric, does not result in abnormally small offspring in one cross, further suggesting that female investment in embryos can be promoted, but not reduced, through embryo-mediated male manipulation/female resistance. However, since the breakdown of co-adapted gene expression can result in similar patterns, we cannot distinguished between gene coadaptation disruption and sexual conflict so far.

Acknowledgements: We thank O. de Gasperín and E. Ávila Luna for help in collecting and maintaining the fish. We also thank to Doctorado en Ciencias Biomédicas (UNAM), the program where YSL is enrolled under the supervision of CMG, and to CONACyT for the scholarship (351769) given to YSL.

References

Abrahamson, W.G. & Gadgil, M., 1973. Growth Form and Reproductive Effort in Goldenrods (Solidago, Compositae). *The American Naturalist,*, 107(957), pp.651–661.

Alleman, M. & Doctor, J., 2000. Genomic imprinting in plants: observations and evolutionary implications. *Plant molecular biology*, 43, pp.147–161.

Arnqvist, G. & Rowe, L., 2002. Antagonistic coevolution between the sexes in a group of insects. *Nature*, 415, pp.787–789.

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

Blackburn, D.G., 2015. Evolution of vertebrate viviparity and specializations for fetal nutrition: A quantitative and qualitative analysis. *Journal of Morphology*, 276(8), pp.961–990.

Blackburn, D.G., 1999. Viviparity and oviparity: Evolution and reproductive strategies. *Encyclopedia of Reproduction*, 4, pp.994–1003.

Bleu, J. et al., 2012. Experimental litter size reduction reveals costs of gestation and delayed effects on offspring in a viviparous lizard. *Proceedings of the Royal Society B: Biological Sciences*, 279(1728), pp.489–498.

Burley, N., 1986. Comparison of the band-colour preferences of two species of estrildid finches. *Animal Behaviour*, 34(6), pp.1732–1741.

Burley, N., 1988. The Differential-Allocation Hypothesis: An Experimental Test. *American Society of Naturalists*, 132(5), pp.611–628.

Chapman, T. et al., 2003. Sexual conflict. *Trends in Ecology & Evolution*, 18(1), pp.41–48.

Constância, M. et al., 2002. Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature*, 417(6892), pp.945–948.

Crespi, B. & Semeniuk, C., 2004. Parent-offspring conflict in the evolution of vertebrate reproductive mode. *Am Nat*, 163(5), pp.635–53.

DeChiara, T.M., Efstratiadis, A. & Robertson, E.J., 1990. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature*, 345, pp.78–80.

DeChiara, T.M., Robertson, E.J. & Efstratiadis, A., 1991. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell*, 64(4), pp.849–859.

De Gasperin, O. & Macías Garcia, C., 2014. Congenital predispositions and early social experience determine the courtship patterns of males of the Amarillo fish. *Behavioral Ecology and Sociobiology*, 68(4), pp.639–648.

Gesundheit, P. & Macias-Garcia, C., 2005. Biogeografía cladística de la familia Goodeidae (Cyprinodontiformes). In J. Llorente-Bousquets & J. Morrone, eds. *Regionalización biogeográfica en Iberoamérica y tópicos afines*. Ciudad de México: Facultad de Ciencias, UNAM, pp. 319–338.

Gil, D. et al., 1999. Male Attractiveness and Differential Testosterone Investment in Zebra Finch Eggs. *Science*, 286(5437), pp.126–128.

González Zuarth, C. & Macías Garcia, C., 2006. Phenotypic differentiation and pre-mating isolation between allopatric populations of Girardinichthys multiradiatus. *Proceedings. Biological sciences / The Royal Society*, 273(1584), pp.301–7.

Greven, H. & Grossherr, M., 1992. Adelphophagy and oophagy in Ameca splendens Miller

and Fitzsimons, 1971. *Zeitschrift fuer Fischkunde*, 12, pp.193–197.

Griggio, M., Morosinotto, C. & Pilastro, a., 2009. Nestlings' carotenoid feather ornament affects parental allocation strategy and reduces maternal survival. *Journal of Evolutionary Biology*, 22, pp.2077–2085.

Haig, D., 2000. The Kinship Theory of Genomic Imprinting. *Annual Review of Ecology and Systematics*, (34), pp.9–32.

Haig, D. & Westoby, M., 1989. Parent-Specific Gene Expression and the Triploid Endosperm. *The American Naturalist*, 134(1), p.147.

Haldane, J.B.S., 1922. Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics*, 12(2), pp.101–109.

Keller, L.F. & Waller, D.M., 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution*, 17(5), pp.230–241.

Lessells, C.M. & McNamara, J.M., 2012. Sexual conflict over parental investment in repeated bouts: negotiation reduces overall care. *Proceedings of the Royal Society B: Biological Sciences*, 279(1733), pp.1506–14.

Lombardi, J. & Wourms, J.P., 1988. Embryonic growth and trophotaenial development in goodeid fishes (Teleostei: Atheriniformes). *Journal of Morphology*, 197(2), pp.193–208.

Lombardi, J. & Wourms, J.P., 1985a. The trophotaenial placenta of a viviparous goodeid fish. I: Ultrastructure of the Internal Ovarian Epithelium, the Maternal Component. *Journal of molecular biology*, 184, pp.277–292.

Lombardi, J. & Wourms, J.P., 1985b. The trophotaenial placenta of viviparous goodeid fish. II. Ultrastructure of the trophotaenia, the embryonic component. *Journal of Morphology*, 184(3), pp.293–309.

Macias-Garcia, C., Jiménez, G. & Contreras, B., 1994. Correlational evidence of a sexually-selected handicap. *Behavioral Ecology and Sociobiology*, 35, pp.253–259.

Macías-Garcia, C. & Saborío, E., 2004. Sperm competition in a viviparous fish. *Environmental Biology of Fishes*, 70(3), pp.211–217.

Macías Garcia, C., 2014. Mode of Reproduction, Mate Choice, and Species Richness in Goodeid Fish. In R. H. Macedo & G. Machado, eds. *Sexual Selection: Perspectives and Models from the Neotropics*. San Diego: Academic Press, pp. 253–288.

Macías Garcia, C. et al., 2012. Variation in sexual dimorphism and assortative mating do not predict genetic divergence in the sexually dimorphic Goodeid fish Girardinichthys multiradiatus. *Current Zoology*, 58(3), pp.440–452.

Macías Garcia, C., Saborío, E. & Berea, C., 1998. Does male-biased predation lead to male scarcity in viviparous fish? *Journal of Fish Biology*, 53(supl. A), pp.104–117.

Magurran, A.E. & Seghers, B.H., 1994. A Cost of Sexual Harassment in the Guppy, Poecilia reticulata. *Proc. R. Soc. Lond. B*, 258(1351), pp.89–52.

Marshall, D.J., Bonduriansky, R. & Bussière, L.F., 2008. Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. *Ecology*, 89(9), pp.2506–2517.

Maynard Smith, J., 1977. Parental investment: A prospective analysis. *Animal Behaviour*, 25(PART 1), pp.1–9.

McNamara, J.M. et al., 2003. Should young ever be better off with one parent than with two? *Behavioral Ecology*, 14(3), pp.301–310.

Miles, D.B., Sinervo, B. & Frankino, W.A., 2000. Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards. *Evolution*, 54(4), pp.1386–1395.

Moore, T. & Haig, D., 1991. Genomic imprinting in mammalian development: a parental tug-of-war. *Trends in Genetics*, 7(2), pp.45–49.

O'Neill, M.J. et al., 2000. Allelic expression of IGF2 in marsupials and birds. *Development genes and evolution*, 210, pp.18–20.

O'Neill, M.J. et al., 2007. Ancient and continuing Darwinian selection on insulin-like growth factor II in placental fishes. *Proceedings of the National Academy of Sciences of the United States of America*, 104(30), pp.12404–12409.

Oldroyd, B.P. et al., 2014. A parent-of-origin effect on honeybee worker ovary size. *Proceedings of the Royal Society B: Biological Sciences*, 281(1775), p.20132388.

Ortíz-Barrientos, D., Counterman, B.A. & Noor, M.A.F., 2007. Gene expression divergence and the origin of hybrid dysfunctions. *Genetica*, 129(1), pp.71–81.

Osorno, J.L. & Székely, T., 2004. Sexual conflict and parental care in magnificent frigatebirds: Full compensation by deserted females. *Animal Behaviour*, 68(2), pp.337–342.

Parker, A.G.A., 1974. Courtship Persistence and Female-Guarding as Male Time Investment Strategies. *Behaviour*, 48(1), pp.157–184.

Parker, G.A., 2006. Sexual conflict over mating and fertilization: an overview. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 361(1466), pp.235–259.

Parker, G.A., 1979. Sexual selection and sexual conflict. In M. Blum & N. Blum, eds. *Sexual Selection and Reproductive Competition in Insects*. New York: New York Academy Press, pp. 123–166.

Parker, G.A. & Partridge, L., 1998. Sexual conflict and speciation. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 353(1366), pp.261–74.

Reznick, D., 1982. Genetic determination of offspring size in the guppy (Poecilia reticulata). *American Naturalist*, 120(2), pp.181–188.

Reznick, D., 1981. "Grandfather Effects": The Genetics of Interpopulation Differences in Offspring Size in the Mosquito Fish. *Evolution*, 35(5), pp.941–953.

Rice, W.R. & Holland, B., 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behav Ecol and Sociobiol*, 41, pp.1–10.

Ritchie, M.G. et al., 2005. Patterns of speciation in endemic Mexican Goodeid fish: Sexual conflict or early radiation? *Journal of Evolutionary Biology*, 18(4), pp.922–929.

Ritchie, M.G. et al., 2007. Sex and differentiation: Population genetic divergence and sexual dimorphism in Mexican goodeid fish. *Journal of Evolutionary Biology*, 20, pp.2048–2055.

Rogers, J.F. & Dawson, W.D., 1970. Foetal and placental size in a Peromyscus species cross. *J Reprod Fertil*, 21(2), pp.255–262.

Rowe, L., Cameron, E. & Day, T., 2003. Detecting sexually antagonistic coevolution with population crosses. *Proc. R. Soc. Lond. B*, 270, pp.2009–2016.

Schindler, J., 2005. Digestion of embryotrophic protein in Goodeid teleosts. In M. C. Uribe

& H. J. Grier, eds. *Viviparous fishes*. Florida: New Life Publication, pp. 266–267.

Schrader, M. & Travis, J., 2009. Do Embryos Influence Maternal Investment? Evaluating Maternal-Fetal Coadaptation and the Potential for Parent-Offspring Conflict in a Placental Fish. *Evolution*, 63(11), pp.2805–2815.

Schrader, M. & Travis, J., 2008. Testing the viviparity-driven-conflict hypothesis: parent-offspring conflict and the evolution of reproductive isolation in a poeciliid fish. *The American naturalist*, 172(6), pp.806–817.

Seigel, R.A., Huggins, M.M. & Ford, N.B., 1987. Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia*, 73(4), pp.481–485.

Shikano, T., Nakadate, M. & Fujio, Y., 2000. An experimental study on strain combinations in heterosis in salinity tolerance of the guppy Poecilia reticulata. *Fisheries Science*, 66(4), pp.625–632.

Shull, G.H., 1908. The composition of a field of Maize. *Journal of Heredity*, 4(1), pp.296–301.

Smith, M.L., 1980. *The evolutionary and ecological history of the fish fauna of the Rio Lerma basin, Mexico*. University of Michigan.

Smuts, B.B. & Smuts, R.W., 1993. Male Aggression and Sexual Coercion of Females in Nonhuman Primates and Other Mammals: Evidence and Theoretical Implications. *Advances in the Study of Behavior*, 22, pp.1–63.

Stearns, S., 1992. *The evolution of life histories*, New York: Oxford University Press.

Templeton, A.R. et al., 1986. Local Adaptation , Coadaptation , and Population Boundaries. *Zoo Biology*, 5, pp.115–125.

Tilghman, S.M., 1999. The sins of the fathers and mothers: Genomic imprinting in mammalian development. *Cell*, 96(2), pp.185–193.

Trexler, J.C. & DeAngelis, D.L., 2003. Resource allocation in offspring provisioning: An evaluation of the conditions favoring the evolution of matrotrophy. *American Naturalist*, 162(5), pp.574–585.

Trivers, R.L., 1974. Parent-Offspring Conflict. *American Zoologist*, 14(1), pp.249–264.

Trivers, R.L., 1972. Parental Investment and Sexual Selection. In *Selection & the Descent of Man, , New York*. New York: Aldine de Gruyter, pp. 134–179.

Uribe, M.C. & García Alarcón, A., 2005. The ovary of viviparous teleosts. Morphological differences between the ovaries of Goodea atripinnis and Ilydon whitei (Goodeidae). In M. C. Uribe & H. J. Grier, eds. *Viviparous fishes*. Florida: New Life Publication, pp. 217–235.

Velando, A., Beamonte-Barrientos, R. & Torres, R., 2006. Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. *Oecologia*, 149(3), pp.535–542.

Webb, S.A. et al., 2004. Molecular phylogeny of the livebearing Goodeidae (Cyprinodontiformes). *Molecular Phylogenetics and Evolution*, 30(3), pp.527–544.

Wilkins, J.F. & Haig, D., 2003. What good is genomic imprinting: the function of parent-specific gene expression. *Nature Reviews Genetics*, 4(5), pp.359–368. Wolf, J.B., 2013. Evolution of genomic imprinting as a coordinator of coadapted gene expression. *Proceedings of the National Academy of Sciences*, 110(13), pp.5085–5090.

Wolf, J.B. & Brodie III, E.D., 2009. The Coadaptation of Parental and Offspring Characters.

Evolution, 52(2), pp.299–308.

Wolf, J.B., Oakey, R.J. & Feil, R., 2014. Imprinted gene expression in hybrids: perturbed mechanisms and evolutionary implications. *Heredity*, 113(2), pp.167–75.

Wourms, J.P., Grove, B. & Lombardi, J., 1988. The maternal-embryonic relationship in viviparous fishes. In W. Hoar & D. Randall, eds. *Fish Physiology*. New York: Academic Press, pp. 1–134.

Zeh, D.W. & Zeh, J. a, 2000. Reproductive mode and speciation: the viviparity-driven conflict hypothesis. *BioEssays*, 22(10), pp.938–46.

Table 1. Variables measured from the mothers and offspring upon birth (except survival, which is the proportion of members of each brood that survived to age = 4 days). Standard length (SL) and width (W) are reported in millimeters; mass is expressed in grams; brood size is the total number of fish born, and reproductive allocation (RA) is the ratio of total brood mass / brood + mother mass. One female gave birth to only dead fish; she was not measured and data on her brood were included exclusively in the analyses of brood size and of offspring. Discrepancies in sample size of female variables were due to female mortality after birth but before all her measures were taken (see Materials and Methods). Eight broods were composed of dead fish that afforded no reliable estimates of offspring size and/or mass.

	Cross											
	M-M			M-Z			Z-M			Z-Z		
Variable	X	SD	N	X	SD	N	X	SD	N	X	SD	N
Mother												
SL	34.05	5.51	15	34.79	3.68	11	30.32	3.83	17	29.05	4.18	29
W	8.82	1.60	15	9.35	1.27	11	7.68	1.35	17	7.27	1.11	29
Mass	0.56	0.21	16	0.54	0.16	11	0.36	1.36	17	0.32	0.17	29
Brood size	6.10	4.46	19	5.54	3.38	13	5.72	3.91	18	6.59	3.12	30
RA	0.14	0.08	16	0.15	0.07	11	0.17	0.08	17	0.14	0.06	29
Mean offspring												
SL	11.20	1.28	16	11.73	0.78	11	11.36	1.13	17	10.3	1.11	28
W	2.55	0.50	16	2.69	0.29	11	2.56	0.34	17	2.25	0.36	28
Mass	0.17	0.006	16	0.18	0.005	11	0.15	0.008	17	0.10	0.005	29
Survival	0.67	0.43	19	0.58	0.41	13	0.89	0.25	18	0.79	0.28	30
Largest offspring												
SL	12.08	1.68	16	12.57	0.97	11	12.46	1.28	17	11.26	1.40	28
W	2.64	0.59	16	2.87	0.40	11	2.79	0.51	17	2.45	0.48	28

Figure 1. a) Length (SL), b) width, and c) weight of the offspring from intra- and interpopulation crosses of adult *G. multiradiatus*. Fish used for these crosses were the laboratory raised F1 generation from San Matías (M) and Zempoala (Z). Significant interactions between paternal and maternal (X-axis) origin as those seen in (a) and (b) are predicted when there is sexual conflict over parental provisioning of embryos.

Figure 2. a) Reproductive allocation (RA; brood weight / brood + mother weight) by female G*. multiradiatus* from San Matías (M) and Zempoala (Z) seemed unaffected by the population of origin of their partner, but the positive correlation between female size (SL) and RA, evident in intrapopulation crosses (only marginally significant in M-M) was absent in interpopulation crosses (b), suggesting that the interaction with the male genotype disrupted the pattern of resource allocation by females from both localities. Females from Zempoala produced larger broods irrespective of male origin (c), and in general brood size was a positive function of female size, but this pattern disappeared when females from San Matías were mated with Zempoala males (d) again suggesting that the interaction with foreign male genotype disrupted the investment on offspring by females from San Matías.

Figure 2. a) Reproductive allocation by female *G. multiradiatus* from San Matías (M) and Zempoala (Z) was the same when mated to Zempoala, but not to San Matias males (the effect was not significant after Bonferroni; see below). Zempoala females produced larger broods irrespective of male origin (b). Graphs show means adjusted for female size (SL).

Figure 1.

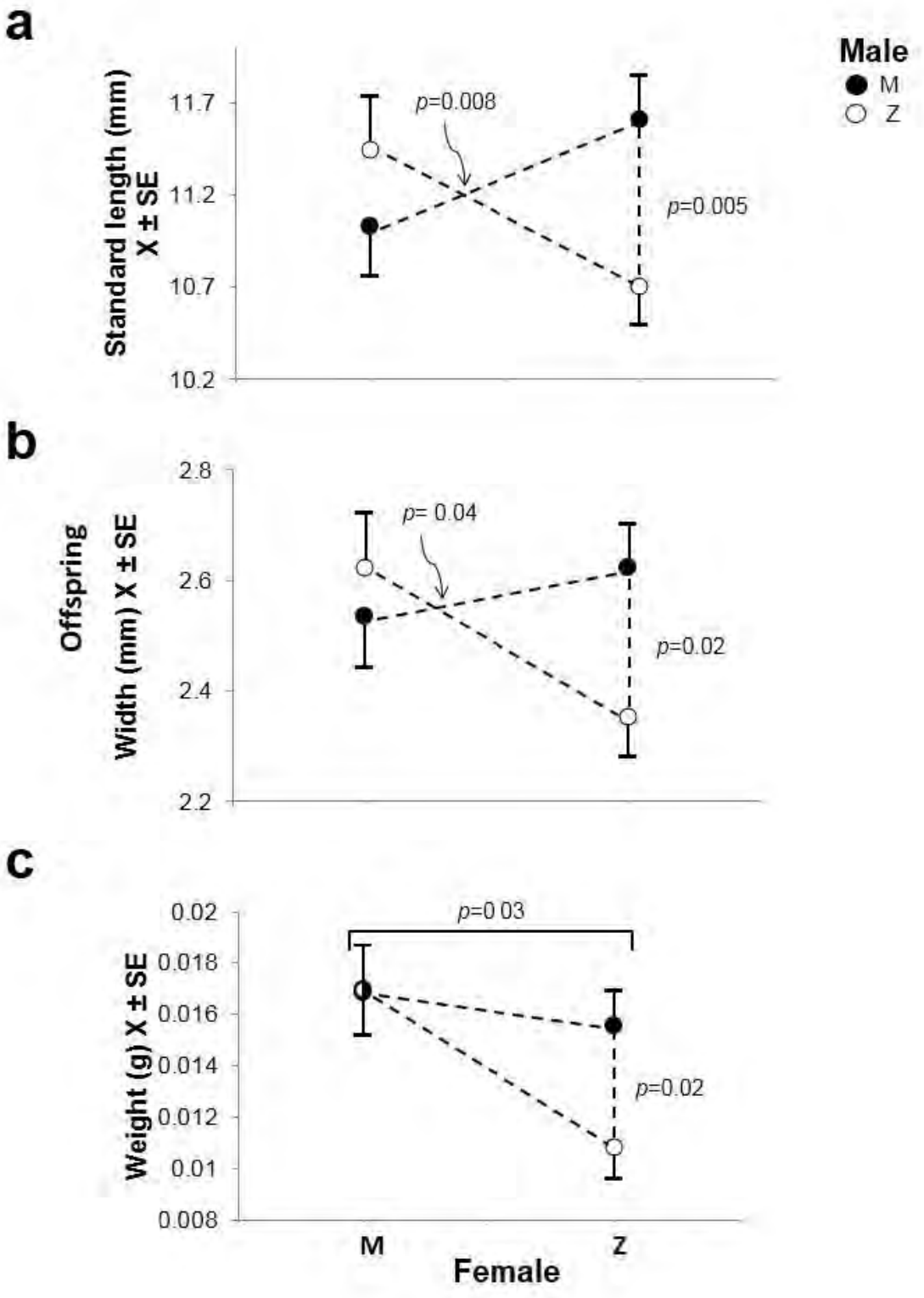
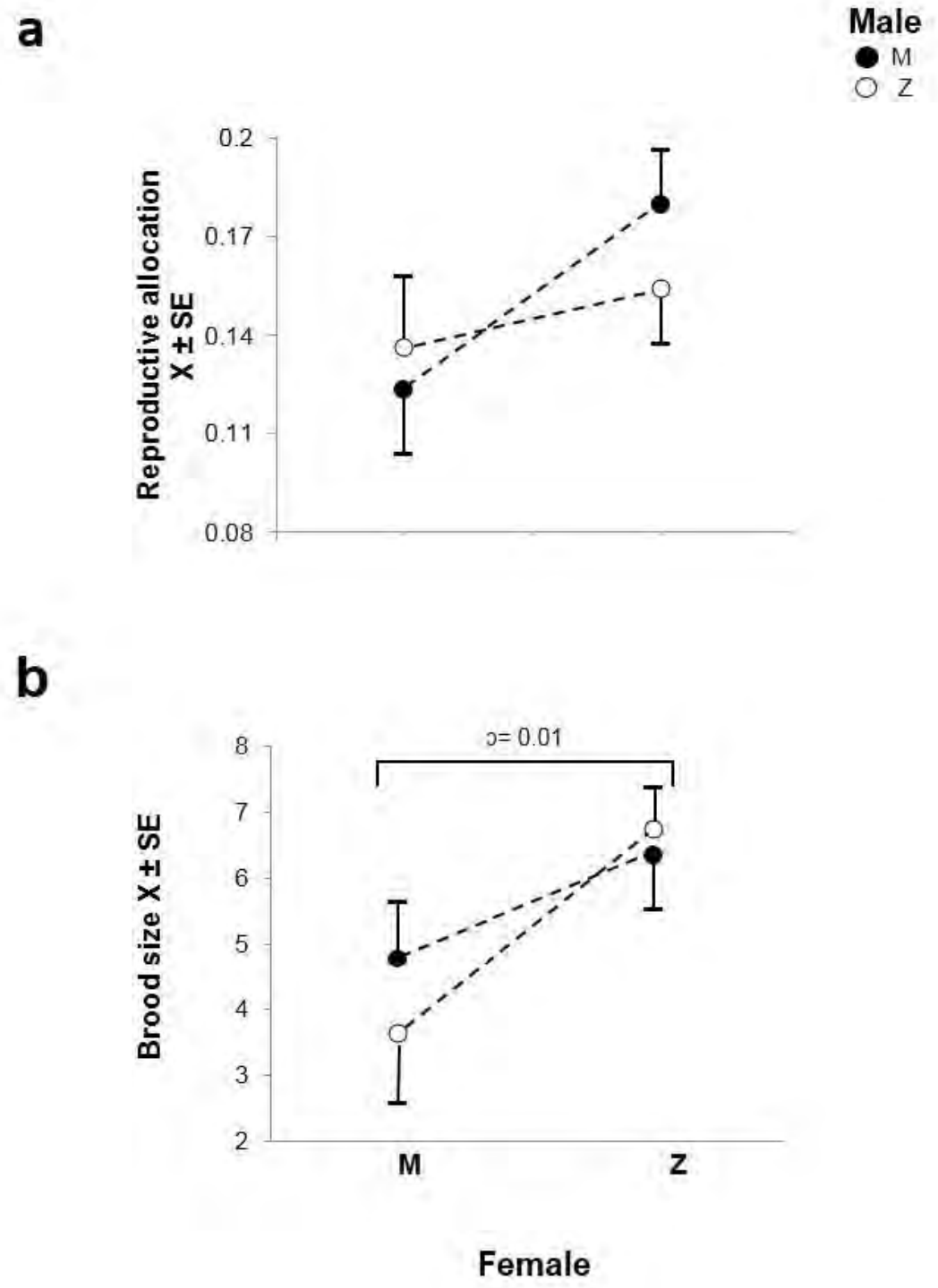

a
Standard length (mm) X ± SE
11.7
11.2
10.7
10.2
p=0.008
p=0.005
Male
M
Z
b
Offspring Width (mm) X ± SE
2.8
2.6
2.4
2.2
p= 0.04
p=0.02
c
Weight (g) X ± SE
0.02
0.018
0.016
0.014
0.012
0.01
0.008
p=0 03
p=0.02
M
Z
Female

Figure 2.


a
Male
M
Z
Reproductive allocation
X ± SE
0.2
0.17
0.14
0.11
0.08
b
p= 0.01
Brood size X ± SE
8
7
6
5
4
3
2
M
Z
Female

Appendix

Table S1

Output from the Generalized Mixed Model (GML) used to evaluate differences in offspring standard length (SL) between crosses. Mother = population of origin of the mother, Father = population of origin of the father, SL = Standard Length, M = San Matías, Z = Zempoala. Data obtained from the output generated by NCSS 2007 v.7.1.21.

Term-by-Term Hypothesis Test Results

Model Term	*F*-Value	Num. *df*	Denom. *df*	*p* - Level
Female SL	27.69	1	62.6	0.000002
Mother	0.04	1	66.4	0.84
Father	1.17	1	66.6	0.28
Mother*Father	6.96	1	65.6	0.01

Name Intercept Mother*Father	Mean	Standard Error of Mean
M, M	11.06	0.25
M, Z	11.43	0.29
Z, M	11.64	0.23
Z, Z	10.75	0.19

Individual Comparison Hypothesis Test Results
Covariates: Female SL=32.7863

Comparison/ Covariate(s)	Comparison Mean Difference	*F*-Value	Num.. *df*	Denom.. *df*	Raw *p* - Level	Bonferroni *p* - Level
Mother		0.04	1	66.4	0.84	
Mother: M - Z	0.06	0.04	1	66.4	0.84	0.84 [1]
Father		1.17	1	66.6	0.28	
Father: M - Z	0.26	1.17	1	66.6	0.28	0.28 [1]
Mother*Father		6.96	1	65.6	0.01	
Mother = M, Father: M - Z	-0.37	0.96	1	67.0	0.33	0.66 [2]
Mother = Z, Father: M - Z	0.89	9.5	1	64.5	0.003	0.006 [2]
Father = M, Mother: M - Z	-0.58	2.64	1	68.0	0.11	0.22 [2]
Father = Z, Mother: M - Z	0.69	3.54	1	64.3	0.06	0.13 [2]

These *F*-Values test Type-III (adjusted last) hypotheses.

Report Definitions
Comparison/Covariate(s): Illustrates the comparison being made.
Comparison Mean Difference: The difference in means for each comparison.
F-Value: The test statistic value corresponding to the L matrix used for testing the model term.
Num *DF*: The numerator degrees of freedom for the *F*-test.
Denom *DF*: The denominator degrees of freedom for the *F*-test.
Raw Prob Level: Gives the strength of evidence for a single comparison, unadjusted for multiple testing.
Bonferroni Prob Level: Gives the p-value adjusted to multiple tests. The number in brackets (e.g. [4]) denotes the number of tests for which the raw prob level was adjusted.

Table S2

Output from the Generalized Mixed Model (GML) used to evaluate differences in offspring width between crosses. Mother = population of origin of the mother, Father = population of origin of the father, SL = Standard Length, M = San Matías, Z = Zempoala. Data obtained from the output generated by NCSS 2007 v.7.1.21.

Term-by-Term Hypothesis Test Results

Model Term	*F*-Value	Num. *df*	Denom. *df*	*p* - Level
Female SL	18.11	1	63.6	0.00007
Mother	0.82	1	67.0	0.37
Father	1.3	1	67.0	0.26
Mother*Father	4.15	1	66.3	0.05

Name	Mean	Standard Error of Mean
Intercept		
Mother*Father		
M, M	2.55	0.09
M, Z	2.62	0.10
Z, M	2.63	0.08
Z, Z	2.37	0.07

Individual Comparison Hypothesis Test Results
Covariates: Female SL=32.7863

Comparison/ Covariate(s)	Comparison Mean Difference	*F*-Value	Num. *df*	Denom. *df*	Raw *p* - Level	Bonferroni *p* - Level
Mother		0.82	1	67.0	0.37	
Mother: M - Z	0.08	0.82	1	67.0	0.37	0.37 [1]
Father		1.3	1	67.0	0.26	
Father: M - Z	0.09	1.3	1	67.0	0.26	0.26 [1]
Mother*Father		4.13	1	66.3	0.05	
Mother = M, Father: M - Z	-0.07	0.32	1	67.7	0.57	1.00 [2]
Mother = Z, Father: M - Z	0.26	6.89	1	64.8	0.01	0.02 [2]
Father = M, Mother: M - Z	-0.08	0.48	1	68.2	0.49	0.98 [2]
Father = Z, Mother: M - Z	0.25	3.99	1	65.3	0.05	0.1 [2]

These *F*-Values test Type-III (adjusted last) hypotheses.

Report Definitions
Comparison/Covariate(s): Illustrates the comparison being made.
Comparison Mean Difference: The difference in means for each comparison.
F-Value: The test statistic value corresponding to the L matrix used for testing the model term.
Num *DF*: The numerator degrees of freedom for the *F*-test.
Denom *DF*: The denominator degrees of freedom for the *F*-test.
Raw Prob Level: Gives the strength of evidence for a single comparison, unadjusted for multiple testing.
Bonferroni Prob Level: Gives the p-value adjusted to multiple tests. The number in brackets (e.g. [4]) denotes the number of tests for which the raw prob level was adjusted.

Table S3

Output from the Generalized Mixed Model (GML) used to evaluate differences brood size between crosses. Mother = population of origin of the mother, Father = population of origin of the father, SL = Standard Length, M = San Matías, Z = Zempoala. Data obtained from the output generated by NCSS 2007 v.7.1.21.

Term-by-Term Hypothesis Test Results

Model Term	*F*-Value	Num. *df*	Denom. *df*	*p* - Level
Female SL	21.73	1	67.0	0.00001
Mother	5.4	1	67.0	0.02
Father	0.08	1	67.0	0.77
Mother*Father	0.53	1	67.0	0.47

Name Intercept Mother*Father	Mean	Standard Error of Mean
M, M	4.60	0.89
M, Z	3.76	1.05
Z, M	6.17	0.81
Z, Z	6.54	0.65

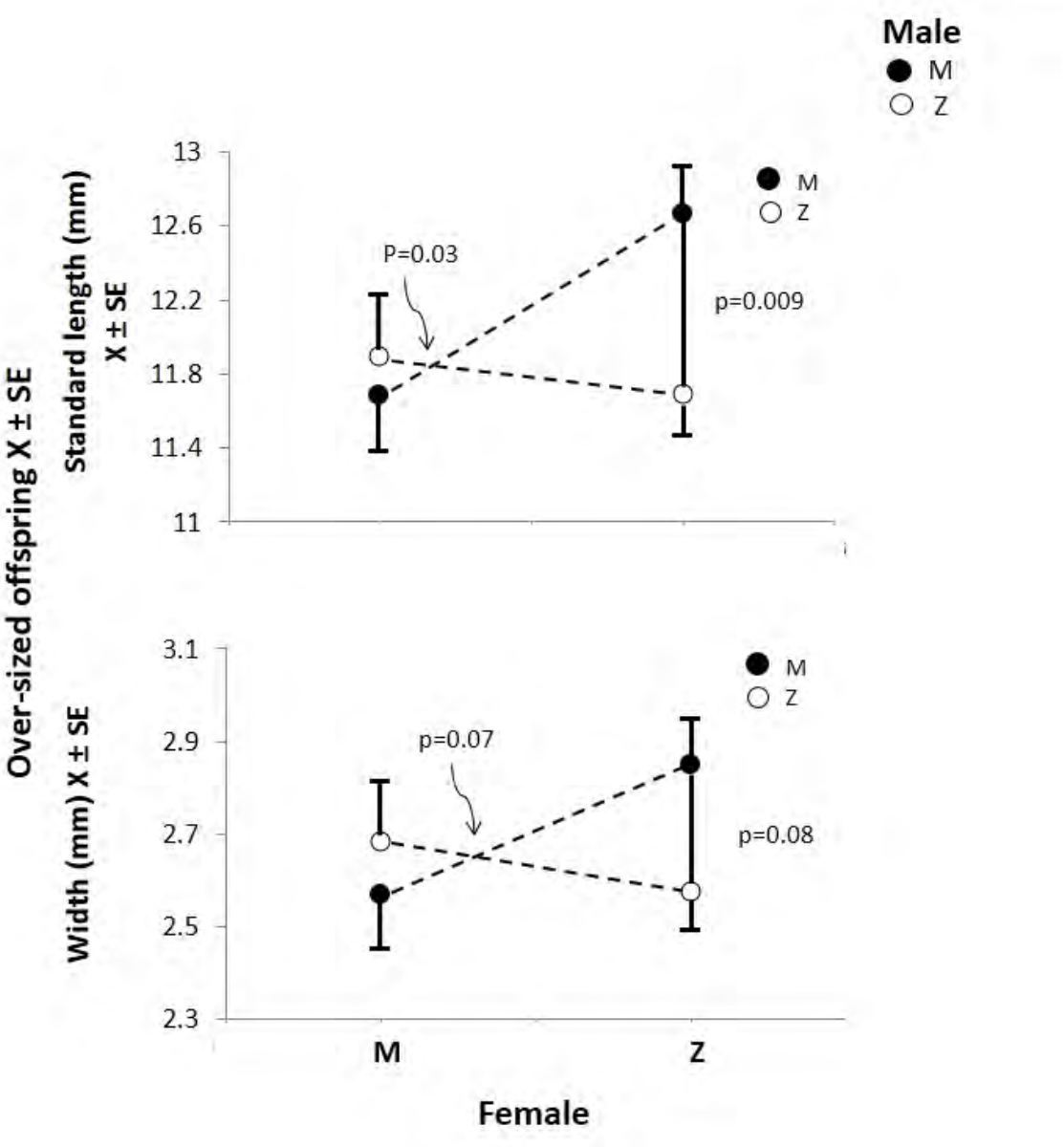
Individual Comparison Hypothesis Test Results
Covariates: Female SL=31.2661

Comparison/ Covariate(s)	Comparison Mean Difference	*F*-Value	Num. *df*	Denom. *df*	Raw *p* - Level	Bonferroni *p* - Level
Mother		5.4	1	67.0	0.02	
Mother: M - Z	-2.18	5.4	1	67.0	0.02	0.02[1]
Father		0.08	1	67.0	0.77	
Father: M - Z	0.24	0.08	1	67.0	0.77	0.77[1]
Mother*Father		0.53	1	67.0	0.47	
Mother = M, Father: M - Z	0.85	0.41	1	67.0	0.52	1.00[2]
Mother = Z, Father: M - Z	-0.37	0.13	1	67.0	0.719	1.00[2]
Father = M, Mother: M - Z	-1.57	1.65	1	67.0	0.2	0.41[2]
Father = Z, Mother: M - Z	-2.79	4.68	1	67.0	0.03	0.07[2]

These *F*-Values test Type-III (adjusted last) hypotheses.

Report Definitions
Comparison/Covariate(s): Illustrates the comparison being made.
Comparison Mean Difference: The difference in means for each comparison.
F-Value: The test statistic value corresponding to the L matrix used for testing the model term.
Num *DF*: The numerator degrees of freedom for the *F*-test.
Denom *DF*: The denominator degrees of freedom for the *F*-test.
Raw Prob Level: Gives the strength of evidence for a single comparison, unadjusted for multiple testing.
Bonferroni Prob Level: Gives the p-value adjusted to multiple tests. The number in brackets (e.g. [4]) denotes the number of tests for which the raw prob level was adjusted.

Figure S1. a) Length (SL) and b) width of the largest newborn from each brood from intra- and interpopulation crosses of adult *G. multiradiatus*. Fish used for these crosses were the laboratory raised F1 generation from San Matías (M) and Zempoala (Z). The significant interactions between paternal and maternal populations of origin are suggestive of sexual conflict over provisioning of embryos in this viviparous matrotrophic fish.



CHAPTER 5

The *Insilin-like growh factor 2*, a very important gene during offspring development, is under positive Darwinian selection in teleosts placental fishes, but how conserved is among the Goodeinae and between Z and M populations of *G. multiradiatus*?

El factor de crecimiento tipo insulin 2 de los goodeinos

The insulin-like growth factor 2 of goodeins

Yolitzi Saldivar Lemus[1*], Jean-Philippe Vielle-Calzada[2], Michael G. Ritchie[3] & Constantino Macías Garcia[1]

[1]Instituto de Ecología, Universidad Nacional Autónoma de México, [2] UGA Laboratorio Nacional de Genómica para la Biodiversidad CINVESTAV Irapuato México, [3]School of Biology, University of St. Andrews.

This manuscript is a more detailed version of the SNP analysis publised in the article of Appendix 5

Abstract. The essential role of the insulin-like growth factor 2 (*igf2*) during embryonic development has lead the evolution of this gene to conserve great proportion of the mRNA or the amino acid sequence identity across unrelated taxa; nevertheless, this gene is under strong positive selection in placental fish as a consequence of mother-offspring conflict over maternal allocation. In species with conflicting mating interests, however, additional evolutionary forces could be playing a role in the evolution of several traits involved in this conflict. When sexual selection is strong on males and thus they face great costs of mating, sexual conflict over several attributes of reproduction (including genes that regulate maternal allocation) may evolve. We sequenced *igf2* of several members of the Goodeidae family that vary in dichromatism and courtship display complexity, attributes that are evolving under sexual selection. As expected, we found that *igf2* identity is much conserved among teleosts. Within goodeins, in spite of the phylogenetic relationships, *igf2* evolution seems to converge among species with stronger sexual selection and among species with weaker sexual selection. Additionally, nucleotide variations within the coding region of this gene appears to be biased to exon 2, which may suggest that this exonic sequence could be at least partially related to a regulatory region of the gene or that it could be influencing gene expression, mRNA conformation or translational efficiency of IGF2. Our findings suggest that *igf2* evolution has been driven by diverse evolutionary forces acting together on its sequence.

Keywords: Sexual selection, sexual conflict, courtship, dichromatism, Goodeinae

Introduction

The insulin-like growth factors (IGFs) are very conserved peptides that are essential in regulating fundamental functions such as growth, development, metabolism and longevity in a wide variety of animals (Wood et al. 2005; Zou et al. 2009).The insulin-like growth factors 1 and 2 (IGF1 and IGF2) are single chain proteins composed of four domains that have a similar structure to proinsulin. Nonetheless, IGF peptides, unlike the proinsulin, maintain the D domain after maturation (D-B-C-A; Wood et al. 2005; Yuan et al. 2011).

The IGF system is composed of ligands (IGF1 and IGF2), receptors (type 1 and type 2) and binding proteins (IGFBP) (Duan 1997). IGF1 essentially mediates many of the growth-promoting effects of growth hormone (GH) during postnatal life (Duan 1997; Robertson 1995). *Igf1* is also expressed during embryogenesis, albeit at a lower level and with a more restricted pattern compared to *Igf2* (Bondy et al. 1990; Streck & Pintar 1992). IGF2, on the contrary, is essential for regulating foetal growth (DeChiara et al. 1990; DeChiara et al. 1991).

Whole-genome duplication events are thought to be responsible for the great diversity and success of the living vertebrates (Dehal & Boore 2005). The evolution of IGFs in fish seems to be the product of several whole-genome duplication events, that first produced insulin and IGFs (1R), a second round (2R) that gave rise to IGF1 and IGF2, and a 3R that originated IGF1a, IGF1b, IGF2a and IGF2b (Zou et al. 2009). Nevertheless, this last duplication that gave rise to *igf2*a and *igf2*b seems to be exclusive to members of the family Cyprinidae. The rest of the fish families only have copy b (Yuan et al. 2011).

The structure of *igf2* appears to be maintained among teleosts. The chum salmon (Palamarchuk et al. 2002), barramundi (Collet et al. 1997), and zebrafish (Ensembl accession number ENSDART00000047569.4) *igf2* genes are composed of four exons and three introns, spanning approximately 7.9, 5.5, and 3.3 kb of genomic DNA, respectively. Although transcription length varies across species, translation length is again preserved

among species (214 aa chum salmon- GenBank accession number CAA65862.1; 215 aa barramundi -GenBank accession number AAB64195.1; and 212 residues zebrafish -Ensembl accession number ENSDART00000047569.4- respectively). Mature IGF2 contains 67 amino acids in humans, 70 amino acids in teleosts, and 68 amino acids in the dogfish shark, and among some of the known teleost sequences, identities range between 85 and 100% (Wood et al. 2005). Several features of some domains of mature IGF2 peptides have been conserved in vertebrates, including fish (Wood et al. 2005). This, and the common roll of *igf2* during embryonic development among vertebrates may suggest that the function of *igf2*, and particularly of those sequences, has been important for a proper embryonic development throughout vertebrate evolution.

The Insulin-like growth factor 2 is under positive (directional) selection in teleosts placental fish (the rate of nonsynonymous substitution –amino acid replacement- was above the neutral mutation rate), and mother-offspring conflict seems to be the force driving this gene evolution. (O'Neill et al. 2007); nonetheless, additional selective pressures could be shaping the evolution of this gene, such as gene conflict over maternal allocation of resources (Haig 2000b), or sexual selection in the form of sexual conflict over several traits related to mating (Gage et al. 2002).

Goodeins are fish that belong to a subfamily of cyprinodontiformes endemic of freshwaters of the Mexican Plateau. Goodenae contains approximately 36 viviparous species distributed in 16 genera (Webb et al. 2004). Goodeins are matrotrophic viviparous fish, which means that embryos develop within the ovarian lumen for approximately 7-8 weeks, where they receive maternal nutrients through the trophotaenial placenta (Lombardi & Wourms 1985a). This organ is constituted by the trophotaenia (embryonic component), a structure that projects from the embryonic hindgut into the fluid-filled ovarian lumen, and by the ovarian lumen (maternal component) (Wourms & Lombardi 1992).

All the species vary in the degree of sexual dimorphism (colour and brightness), and in the complexity of their courtship displays repertoire, which may include many elaborated dances. Mate choice of *Girardinichthys multiradiatus* females is based on size and colour of male dorsal and anal fins, and on courtship intensity (Macias-Garcia et al. 1994; Macías-Garcia & Saborío 2004). Courtship complexity is correlated with speciation ratio in goodeids (Méndez Janovitz 2011), thus it is probably an attribute under sexual selection.

We selected four goodein species for this study: *G. multiradiatus*, *G. viviparous*, *Allotoca diazi* and *Ilyodon furcidens*, which differ in degree of sexual dimorphism, courtship complexity and location within the phylogeny by Webb and co-workers (2004)

The genus *Girardinichthys* comprises only two species: *G. multiradiatus* and *G. viviparus.* These sister species have very contrasting attributes related to mating. Both are sexually dimorphic, where males have bigger and more colourful anal and caudal fins than females; nevertheless, between this two species, there is a large variation in attributes linked to sexual selection. *G. multiradiatus* males have conspicuous yellow and black anal and caudal fins, and show an elaborated repertoire of courtship display, while males of *G. viviparous*, on the contrary, have a cryptic black anal and caudal fin coloration and simple courtship display.

Allotoca diazi belongs to the sister clade of that of *Girardinichthys* (see Webb et al. 2004). This species exhibits weak dichromatism (Méndez Janovitz pers. comm.), but have a complex courtship display (see Méndez Janovitz 2011). *Ilyodon furcidens*, on the other hand, belongs to a distant clade within the phylogeny and it also shows weak dichromatic colouration (Méndez Janovitz pers. comm.),and very simple courtship (see Méndez Janovitz 2011).

We used the DNA, RNA and amino acid linear sequences of *igf2* of these species and two other actinopterygii (one viviparous and one oviparous) to conduct a descriptive analysis on the sequence variation of this genen within the Goodeins. Additionally, we tried to interprete these results from a wider perspective, where several evolutionary forces could have been shaping the evolution of *igf2* (but that could apply to other genes or attributes) of individuals.

Methods

Study Species

Fish used were descendants of individuals collected from the field under SAGARPA permit SGPA/DGVS/01290/13. Fish were kept at the Instituto de Ecología, UNAM under standardised conditions.

We used one male and one female from San Matías el Grande –M- and Zempoala-Z- populations (Michoacán and Estado de México, respectively) of *G. multiradiatus*, one male and one female of *G. viviparous* (Chapultepec population), and one male of *A. diazi* (El Molino de Chapultepec population).

Ilyodon furcidens (Webb et al. 2004) was named as *I. amecae* by O'Neill and co-workers (2007); however, according to the Goodeid Working Group (GWG) the correct name is *I. furcidens*, and therefore from here on we will call *I. furcidens igf2* gene the *I. amecae igf2* sequence reported by O'Neill and co-workers (2007) (GenBank Accession number DQ337453.1).

All methods were carried out in accordance with the Guidelines for the treatment of animals in behavioural research and teaching published by Animal Behaviour (DOI:10.1016/j.anbehav.2011.10.031).

Igf2 sequencing

DNA was isolated using the Qiagen DNeasy Blood & Tissue Kit. Using as a reference the mRNA sequence of *igf2* of *I. furcidens* (GenBank Accession number DQ337453.1), we designed a pair of primers (Table 1) that flank virtually the ca 5 kb Open Reading Frame of *igf2* of the four species, except for the last 17 nucleotides (Figure 1). The PCR reaction contained 16 µl of PCR SuperMix High Fidelity (Invitrogen), 0.5 µl forward primer (50 ng/µl), 0.5 µl reverse primer (50 ng/µl), 1 µl DNA, and was put through a cycling profile of 95 ºC for 5 min, 94 ºC for 30 s, 62 ºC for 30 s, 68 ºC for 5:30 min, 30 cycles, and then 68 ºC for 10 min.

We cloned the PCR fragment using the TOPO TA Cloning Kit (Invitrogen) for electrocompetent cells (TOP10 Electrocomp). Plasmid DNA was extracted using a homemade protocol. We used several overlapping primers to sequence the ca 5 kb fragment (Table 1, Figure 1).

We assembled five sequences of each individual of *G. multiradiatus*, 3 of each fish of *G. viviparus*, and 3 of the *A. diazi* male. We found several SNPs within the sequences, however, for the consensus sequence we normally chose the most frequent nucleotide within the species or population, and when two allelic variations of one SNP were shared by different species or populations, we chose the same for both species/populations. The only exception was one SNP we found in *G. multiradiatus* whose allelic variants were either a thymine (T), found in the Z female, or a guanine (G) present in the Z male; however, since the M male and the M female were also homozygous for the allelic variant T, we used the allelic variant G for the Z sequence, since it produced a change in one amino acid and highlighted the maximum variation among sequences. For the amino acid sequence comparisons, where we used the allelic variant T for the consensus sequence since the T was present in 3 out of 4 *G. multiradiatus* fish.

Sequence analysis

We analysed the sequences of fish from M and Z populations of *G. multiradiatus* and of *G. viviparus* to screen for nucleotide variations, in the form of Single Nucleotide Polymorphisms (SNPs) or Single Nucleotide Variants (SNV), to whom we referred only as SNPs. For this analysis we only considered as an "SNP" in heterozygous individuals when both nucleotides were in a 50-50 ratio, otherwise, they were considered as a polymerase or sequencing errors. Although SNPs are variations in a single nucleotide in the genome of a species, we used the term to refer to either a nucleotide variation in any of the populations of *G. multiradiatus* that was not present in the other or/and in *G. viviparus* or as a nucleotide difference between *G. multiradiatus* and *G. viviparus* sequences.

We used the *igf2* sequences of *I. furcidens* and *Danio rerio* (Ensembl Transcript ID Number *igf2b-001* ENSDART00000047569.4) to determine the exonic and intronic borders of the sequences we assembled. For the analysis of Single Nucleotide Polymorphisms (SNPs), we used the 3 sequences of the two sister species that belonged the genus *Girardinichthys.* We conducted the analysis of gDNA, mRNA and aminoacid identity using the sequences of *D. rerio, I. furcidens*, *G. multiradiatus* (Zempoala and San Matías el Grande Populations), *G. viviparous, A. diazi*, and *Heterandria formosa* (Gene Bank Accession Number AY833403.1).

All the analysis of DNA, mRNA and protein sequences were carried out using the BioEdit (Hall 1999) and CLC Sequence Viewer 7.6 PC Programmes.

Phylogenetic analysis

Phylograms of mRNA and amino acid sequences were obtained using the Phylogeny.fr web service (Dereeper et al. 2008; Dereeper et al. 2010) that performs sequence alignment, curation, phylogeny reconstruction and tree rendering employing Muscle, Gblocks, PhyML and TreeDyn programmes, respectively.

Results

We obtained the virtually complete Open Reading Frame (ORF) sequence of *G. multiradiatus* (Z and M populations)*, G. viviparous and A. diazi.* According to a blast of the coding sequences of *D. rerio, H. formosa* and *I. ameca*, the sequences lacked the last seventeen nucleotides of exón 4 (Figure 2).

Goodein *igf2*, as in several other teleosts, is composed of 4 exons and 3 introns (Figure 2a). The gene length among these species ranges between 4802 and 4814 bp (Table 2, Figure 2b, 2c, 2d, 2e).

Sequence of *igf2* within the *G. multiradiatus'* populations was much conserved. Only 24 SNPs within a 4814-bp sequence were detected and, as expected, most of them were located in intronic regions (Table 3), and therefore don't affect the aminoacid sequence. From those SNPs, five were located within the CDS. Notably, 4 of them were found in Exon 2. Genomic DNA and mRNA sequences shared 99.7% and 99.8% identity respectively, and protein sequences shared 99.5% identity. The mRNA and protein sequences were virtually identical, except for one SNP (guanine –G-) in the sequence of the Z female, that was a thymine (T) in the Z male, M female and M male, and that replaced a leucine (L) for a tryptophan (W) in the Z female aminoacid chain sequence (site 55; Figure 3).

The igf2 sequence is highly conserved within the genus *Girardinichthys*. When comparing *G. multiradiatus* Z population and *G. viviparus igf2* consensus sequences they were also very similar. Genomic DNAs shared 98.6% identity and mRNAs 98.41% identity. The protein sequences were also much conserved (97.1% identity). Additionally, only 2.2% of the gene is polymorphic within the genus *Girardinichthys*. The SNP analysis showed 104 sites that had some degree of variation within a 4818 bp sequence. Between sister species, ninety-one nucleotide variations were found in intronic regions and only 13 in the CDS.

The high similarity between sequences was not exclusive to members of the genus *Girardinichthys*. *Allotoca diazi*, who belongs to its sister clade and therefore also to the group

Girardinichthyini, share 97% of its DNA sequence and 98.4% of its coding sequence (CDS) with Z population. The aminoacid chain was also conserved between *G. multiradiatus* and *A. diazi* with a 97.1% identity, one of the highest similarities among the sequenced we analysed.

Among the four Goodeins we used for this analysis, the two species characterized by a poor courtship display repertoire, *G. viviparus* and *I. furcidens* (Méndez Janovitz 2011), share 97.6% of their CDS and 95.7% of their protein sequence, in spite of being phylogenetically very distant (see Webb et al. 2004). Interestingly, *.G. viviparus'* amino acid sequence share a higher percentage of similarity with *I. furcindens* than with A. *diazi* (95.23%), a species of a sister clade.

More distant species have more diverged genomes (Nosil & Feder 2012). When comparing the CDS of Z population and *Heterandria formosa*, another highly matrotrophic viviparous fish from the same order (Cyprinodontiformes; Pollux et al. 2014), mRNNA sequences shared only 90.64% identity. As expected, this sequence resemblance drops more drastically when comparing mRNA of Z and the oviparous cypriniform *Danio rerio.* Only 73.6% from a 625-bp sequence is shared. The protein sequence variation was similar to mRNA variation. *G. multiradiatus* share 87.6% and 72% of its protein sequence with *H. formosa* and *D. rerio* respectively.

The phylogenetic relationships among goodein species reported by Webb and co-workers (2004) did not coincide with the phylogenetic tree of the amino acid sequence of *igf2* that we obtained. *Girardinichthys multiradiatus* and *A. diazi* were grouped within a clade, and *G. viviparus* and *I. furcidens* were put together within a sister clade (Figure 4). This was probably caused by two amino acids that apparently underwent convergent evolution. The serine (S) of site 146 evolved into a proline (P) after split of the G. multiradiatus and A. diazi clade, and the lysine (K) of site 154 (156 in Figure 3) changed into an asparagine (N)

apparently after the cyprinodontiformes split, and was reverted to the ancestral state by only G. viviparus and I. furcidens (see Figure 4).

Discussion

The Insulin-like growth factor 2 of Goodeins, as in most teleosts, is composed of four exons and three introns (Ensembl Accession ID: spotted gar ENSLOCG00000001806; amazon molly ENSPFOG00000014112; tilapia ENSONIG00000014499; zebrafish ENSDARG00000033307; see also Wood et al. 2005).

It has been estimated that the SNP frequency in the human genome is approximately one in 1 000 bp (Brookes 1999). We found a proportion of SNPs five-fold larger in the ca 5000 bp sequence within *Girardinichthys multiradiatus' igf2* sequence, and twenty-fold bigger within the genus *Girardinichthys*. This, although unexpected, is not quite surprising given the fact that this gene is under positive selection in placental teleosts (O'Neill et al. 2007). Most of nucleotide variation, as expected, was found in intronic regions; nevertheless, exonic regions were much shorter than intronic ones, and when comparing the proportion of SNPs within each region, SNPs distribution was even biased to coding regions in *Girardinichthys multiradiatus* (0.79% of the CDS and 0.45% of the intronic regions) and similar between coding and non-coding regions within the genus (2.06% of the CDS and 2.17% of the non-coding regions). Since introns are not transcribed, changes in the nucleotide sequence don't affect the amino acid sequence, unless they are in a regulatory regions (see Majewski et al. 2002). We don't have any piece of information regarding *igf2* regulation in Goodeins, thus we cannot assess the impact of these SNPs on *igf2* expression; nonetheless, the fact that a major proportion of nucleotide variations in the coding region are located in exon 2 could imply that this region could have an impact on the function of the protein, or could influence gene expression, the mRNA conformation or the translational efficiency (Komar 2003).

We cannot evaluate either the impact of the non-synonymous mutations on IGF2 sequence, structure or function. Some of these SNPs, despite changing the amino acid identity, may not affect the function or stability of the protein, since only 26-32% of natural non-synonymous SNPs have effects on the function of the peptide (Chasman & Adams 2001).

We found evidence suggestive of unexpected convergent evolution of the amino acid sequence of some distant goodeins. As we mentioned above, we don't know the impact of the new amino acid residues on the structure or recognition site of the protein, thus we cannot are affirm whether this substitutions led to convergent evolution of the protein of *G. multiradiatus* and *A. diazi*, and *G. viviparus* and *I. furcidens*; however, this is an approximation that suggests it.

The importance of this gene among vertebrates is highlighted by the strong resemblance among the sequences of completely unrelated species. Sequence identities between human IGF2 and some of the teleosts orthologs range between 70 and 75% (Wood et al. 2005). Furthermore, among distant teleosts that differ in their mode of reproduction, their protein sequences share the same identity range (*G. multiradiatus* vs D. rerio and *H. formosa* vs D. rerio). Nonetheless, although *igf2* has an essential and therefore conserved function for embryonic development in many taxa, it has been subject to positive selection on placental fish species as a result of parent-offspring conflict (O'Neill et al. 2007). We compared the identity of sequences among some Goodeins, analysed their phylogenetic relationships based on their amino acid sequences, and compared those data to sexually selected traits, and our results suggest that, even among species with the same reproductive pattern (placental viviparity), additional evolutionary forces (such as sexual conflict over mating attributes) may also have been shaping the evolution of IGF2.

Courtship is a distinctive trait of almost all goodeins (except for *Ilyodon xantusi)*. Mendez Janovitz (2011) described 21 courting behaviours in this subfamily, which vary in

complexity. The combination and number of courtship displays is exclusive of each species and range from two to six. *Girardinichthys multiradiatus* and *A. diazi* exhibit 6 and 4 courtship displays respectively, while both, *G. viviparus* and *I. furcindens*, have only two (Méndez Janovitz 2011). These data are consistent with dichromatism values, where again, *G. multiradiatus* and *A. diazi* had greater values of dichromatism than *I. furcindens* and *G. viviparus* (Méndez Janovitz unpublished data/pers. com).

Male mating success depends on female acceptance which is based on male ornaments and courtship (Macias-Garcia et al. 1994; Macías-Garcia & Saborío 2004). However, these attributes are costly since males become more conspicuous preys either through a flamboyant colouration or movements (Gonzalez-Zuarth et al. 2010; Macias-Garcia et al. 1994). Yet, courtship is energetically expensive (De Gasperin & Macías Garcia 2014), and conspicuous colourations are immunologically costly to maintain (Arellano-Aguilar & Macías Garcia 2008). Carotenoids, the pigments responsible for yellow, orange and red colourations cannot be self-synthesised, are indicator of the immune response and play an important role in cellular functioning (Bendich & Olson 1988), thus, males must compromise those resources, that are essential for health, on ornaments. Hence, although females face some disadvantages from courtship and conspicuousness (e.g. they become also more notorious if they are next to a colourful courting male), costs of attributes that evolved via sexual selection are biased generally to males, rendering sexual conflict over mating strong in this family.

Conclusions

The insulin-like growth factor gene, in spite of having conserved sequence among vertebrates, seems to have more variation (in the form of SNPs or SNV) than what it would be expected by natural selection. We did not analyse if theses SNPs and nucleotide

variations were synonym or non-synonym mutations, or whether changes in the amino acid sequence altered the structure of the mature peptide, and therefore we cannot assess the functional implications of these variants. Nevertheless, we found evidence suggestive of convergent evolution of the protein sequence within the goodens. Yet, we found some degree of variation within the same genus of a fish in a gene with an essential and conserved function for embryonic development among vertebrates, thus it would be worth to investigate the effect that such nucleotide variation has over *igf2* regulation or protein structure and function within this viviparous family. Additionally, if sexual selection is imposing costs of mating to males and/or females, then such conflict could be driving the evolution of genes, such as *igf2*, in the same direction in species with similar sexually selected attributes.

Acknowledgments

We thank Cesar Álvarez Mejía, Gloria León and Isaac Rodriguez for technical support, and Marcela Méndez Janovitz for providing the data of species dichromatism. Edgar Ávila-Luna helped with the fish husbandry. Research was supported by PAPIIT grant IN216111 (CMG), CONACyT PhD scholarship 351769 (YSL) and the Howard Hughes International Research Program (JPVC). This work was conducted as partial fulfilment of YSL PhD thesis within the Doctorado en Ciencias Biomédicas PhD program (UNAM) under the supervision of CMG.

References

Arellano-Aguilar, O. & Macías Garcia, C., 2008. Exposure to pesticides impairs the expression of fish ornaments reducing the availability of attractive males. *Proceedings. Biological sciences / The Royal Society*, 275(1640), pp.1343–1350.

Bendich, A. & Olson, J.A., 1988. Biological actions of arotenoids. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 3(8), pp.1927–1932.

Bondy, C.A. et al., 1990. Cellular pattern of insulin-like growth factor-I (IGF-I) and type I IGF receptor gene expression in early organogenesis: comparison with IGF-II gene expression. *Molecular endocrinology (Baltimore, Md.)*, 4(9), pp.1386–98.

Brookes, A.J., 1999. The essence of SNPs. *Gene*, 234(2), pp.177–186.

Chasman, D. & Adams, R.M., 2001. Predicting the functional consequences of non-synonymous single nucleotide polymorphisms: structure-based assessment of amino acid variation. *Journal of molecular biology*, 307(2), pp.683–706.

Collet, C. et al., 1997. Organization, sequence, and expression of the gene encoding IGFII from barramundi (teleosteii; Lates calcarifer). *Biochemical Genetics*, 35(7–8), pp.211–224.

DeChiara, T.M., Efstratiadis, A. & Robertson, E.J., 1990. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature*, 345, pp.78–80.

DeChiara, T.M., Robertson, E.J. & Efstratiadis, A., 1991. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell*, 64(4), pp.849–859.

Dehal, P. & Boore, J.L., 2005. Two rounds of whole genome duplication in the ancestral vertebrate. *PLoS Biology*, 3(10).

Dereeper, A. et al., 2010. BLAST-EXPLORER helps you building datasets for phylogenetic analysis. *BMC Evolutionary Biology*, 10(1), p.8.

Dereeper, A. et al., 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic acids research*, 36(Web Server issue), pp.465–469.

Duan, C., 1997. The insulin-like growth factor system and its biological actions in fish. *Amer. Zool.*, 37, pp.491–503.

Gage, M.J.G. et al., 2002. Sexual selection and speciation in mammals, butterflies and spiders. *Proceedings. Biological sciences / The Royal Society*, 269(1507), pp.2309–2316.

De Gasperin, O. & Macías Garcia, C., 2014. Congenital predispositions and early social experience determine the courtship patterns of males of the Amarillo fish. *Behavioral Ecology and Sociobiology*, 68(4), pp.639–648.

Gonzalez-Zuarth, C.A., Vallarino, A. & Macías Garcia, C., 2010. Female responsiveness underlies the evolution of geographic variation in male courtship between allopatric populations of the fish Girardinichthys multiradiatus. *Evolutionary Ecology*, 25(4), pp.831–843.

Haig, D., 2000. The Kinship Theory of Genomic Imprinting. *Annual Review of Ecology and Systematics*, 31(1), pp.9–32.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser*, 41, pp.95–98.

Komar, A.A., 2003. *Single Nucleotide Polymorphisms* Second Edi. A. A. Komar, ed., Cleveland: Humana Press.

Lombardi, J. & Wourms, J.P., 1985. The trophotaenial placenta of a viviparous goodeid fish. I: Ultrastructure of the Internal Ovarian Epithelium, the Maternal Component. *Journal of molecular biology*, 184, pp.277–292.

Macias-Garcia, C., Jiménez, G. & Contreras, B., 1994. Correlational evidence of a sexually-selected handicap. *Behavioral Ecology and Sociobiology*, 35, pp.253–259.

Macías-Garcia, C. & Saborío, E., 2004. Sperm competition in a viviparous fish. *Environmental Biology of Fishes*, 70(3), pp.211–217.

Majewski, J. et al., 2002. Distribution and characterization of regulatory elements in the human genome. *Genome Research*, 12(212), pp.1827–1836.

Méndez Janovitz, M., 2011. *Relación entre la complejidad del cortejo y la tasa de especiación en la subfamilia Goodeinae (Cyprinodontiformes)*. Universidad Nacional Autónoma de México.

Nosil, P. & Feder, J.L., 2012. Genomic divergence during speciation: causes and consequences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1587), pp.332–342.

O'Neill, M.J. et al., 2007. Ancient and continuing Darwinian selection on insulin-like growth factor II in placental fishes. *Proceedings of the National Academy of Sciences of the United States of America*, 104(30), pp.12404–12409.

Palamarchuk, A. et al., 2002. Complete nucleotide sequence of the chum salmon insulin-like growth factor II gene. *Gene*, 295(2 Special Issue SI), pp.223–230.

Pollux, B.J.A. et al., 2014. The evolution of the placenta drives a shift in sexual selection in livebearing fish. *Nature*, 513, pp.233–236.

Robertson, E.J., 1995. Insulin-like growth factors, imprinting and embryonic growth control. *Seminars in Developmental Biology*, 6(4), pp.293–299.

Streck, R.D. & Pintar, J.E., 1992. The embryonic pattern of rat insulin-like growth factor-i gene expression suggests a role in induction and early growth of the liver. *Endocrinology*, 131(4), pp.2030–2032.

Webb, S.A. et al., 2004. Molecular phylogeny of the livebearing Goodeidae (Cyprinodontiformes). *Molecular Phylogenetics and Evolution*, 30(3), pp.527–544.

Wood, A.W., Duan, C. & Bern, H. a., 2005. Insulin-like growth factor signaling in fish. *International Review of Cytology*, 243, pp.215–285.

Wourms, J.P. & Lombardi, J., 1992. Reflections on the evolution of piscine viviparity. *Integrative and Comparative Biology*, 32(2), pp.276–293.

Yuan, X.-N. et al., 2011. Functional conservation and divergence of duplicated insulin-like growth factor 2 genes in grass carp (Ctenopharyngodon idellus). *Gene*, 470(2011), pp.46–52.

Zou, S. et al., 2009. Zebrafish IGF genes: gene duplication, conservation and divergence, and novel roles in midline and notochord development. *PloS one*, 4(9), p.e7026.

Table 1. Sequence of primers used to amplify different fragments of *igf2.*

Primer	Sequence
***igf2* amplification**	
IF_F	5’ ATGGAGACCCAGCAAAGATCCGGAC 3’,
IF_R	5’ AATTGTCTGTGGTGTGCAACACGGC3’
Gene sequencing	
IF_R7	5’ CAGACAAACTGCAGCGCATC 3’
GM_R	5’ GAAATGGCCTCGGCAGAGACGTGGT 3’
GM_F	5’ TTGCTCCAGCAGGTTGAGGTCACAG 3’
GM_F_C	5’ CCGTGGGATTGTAGAAGAGTG 3’
Y_F	5’ ATGAGCTGGGAGGAACGAAA 3’
Y_R	5’ AGG GAG AGG GAG GAC TGA TT 3’
E2_F	5’ TGCTTTAATTTGTGTGTTTCCCC 3’

Table 2. Exonic and intronic lengths of goodein *igf2*. Exon 4 lacks the last 17 nucleotides, but we included them as ≈17 in the second total (left) of Exon 4 length.

Exon/Intron ID	Species			
	G.multiradiatus Z	*G.multiradiatus* M	*G. viviparus*	*A. diazi*
Exon 1	75	75	75	75
Intron 1	810	811	809	811
Exon 2	151	151	151	152
Intron 2	2243	2242	2241	2229
Exon 3	181	181	182	181
Intron 3	1131	1131	1131	1131
Exon 4	223+ ≈17	223+ ≈17	223+ ≈17	223+ ≈17
Total	**4814-4831**	**4814-4831**	**4812-4829**	**4802-4819**

Table 3. SNPs of *igf2* genomic sequence within the genus *Girardinichthys. G.m* denotes individuals of *G. multiradiatus* and *G. v* refers to *G. viviparus*. Z denotes fish from Zempoala population and M fish from San Matías el Grande population.

Location	SNP variants	*G.m*- Z female	*G.m*- Z male	*G.m*- M female	*G.m*- M male	*G.v* female	*G. v* male
I1	C & T	1 C	3 C	4 C	5 C	3 T	3 T
I1	T & C	1 C	1 T, 2 C	4 C	5 C	3 C	3 C
I1	G & T	1 G	3 G	4 G	5 G	3 T	3 G
I1	A & G	1 G	3 G	4 G	5 G	3 A	3 G
I1	A & G	1 A	3 A	4 A	5 A	3 A	2 A, 1 G
I1	G & A	1A	3 A	4 A	5 A	3G	3G
I1	C & -	1 C	3C	4C	5C	3 -	3 -
I1	T & A	1T	3T	4T	5T	3A	3A
I1	C & G	1C	3C	4C	5C	3G	3G
I1	G & C	1G	3G	4G	5G	3C	3C
I1	T & C	1 T	3T	4T	5T	3C	3C
I1	C & T	1C	3C	2C,2T	4C,1T	3C	3C
I1	A, C & T	1A	3A	3A,1C	4A,1C	2A,1T	3A
I1	C, T & A	1T	3T	3T,1C	4T,1C	3A	3A
I1	T & -	1-	3-	4T	5T	3-	3-
I1	T & A	1T	3T	4T	5T	3T	1T,1A
I1	A & G	1A	3A	4A	5A	3A	1A,2G
I1	T & -	1-	3-	4-	5-	3-	3T
I1	T & G	1T	3T	4T	5T	3G	2G
I1	C & T	1C	3C	2T	3C	3C	3C
I1	C & G	1C	ND	ND	2C	3G	3G
I1	T & G	1T	3T	4T	5T	3T	3G
I1	T & A	1A	3A	4A	5A	3A	2T
I1	T, G & C	1T	3T	4G	2T,1G	3T	2T,1C
I1	C & A	1C	3C	ND	5C	3C	3A
E2	A & C	1A	3A	3C	4A	3A	1A,2C
E2	G & T	5G	3T	5T	5T	3T	3T
E2	C, G & T	2C	2C,1G	5C	5C	3T	3T
E2	T & C	1T,1C	3T	5T	5T	3T	3T
E2	C & T	2C	3C	5C	5C	3C	2C,1T
I2	Ts sequence	The number of Ts varies according to the species, population and individual clone					
I2	C & -	2-	3-	5-	4 –,1C	3C	3C
I2	C & T	2C	3C	5C	5C	3C	2C,1T
I2	T & C	2T	3T	5T	5T	3T	2T,1C
I2	T & C	5T	3T	5T	5T	3C	3C
I2	T & C	5T	3T	5T	5T	3C	3C
I2	GA & --	5 GA	3 GA	4 GA	5 GA	3-	3-
I2	AG & --	5 AG	3 AG	3 AG	5 AG	1 AG,2--	2 AG,1 --
I2	C & T	5C	3C	2C,1T	5C	3C	3C
I2	AC & --	5--	3--	3--	5--	3AC	3AC
I2	A & G	5A	3A	5A	5A	2A	2A,1G
I2	G & A	5G	3G	5G	5G	2A	3A

I2	GA & --	4 GA,1--	3 GA	5--	5--	2--	2--
I2	A & T	5A	3A	5A	5A	3T	3T
I2	T & C	5T	3T	5T	5T	3C	3C
I2	A & G	5A	2A,1G	5A	5A	3A	3A
I2	A & T	5A	3A	5A	5A	3T	2T
I2	A & C	5A	3A	5A	5A	3C	3C
I2	G & A	5G	3G	5G	5G	3A	3[a]
I2	G & A	5G	3G	5T	5G	3G	3G
I2	T & A	5T	3T	5T	5T	3T	2T,1A
I2	A & G	5A	3A	5A	5A	3G	3G
I2	T & C	5T	3T	5T	5T	3C	3C
I2	C & T	5C	3C	5C	5C	3T	3T
I2	T & C	5T	3T	5T	5T	3C	3C
I2	T & G	5T	3T	5T	5T	3G	3G
I2	C & T	5C	3C	5C	5C	3T	3T
I2	T & G	5T	3T	5G	5G	3T	3T
I2	T & C	5T	3T	5T	5T	3T	2T,1C
I2	G & -	5G	3G	5G	5G	3-	3-
I2	A & G	5A	3A	5A	5A	3A	2A,1G
I2	T & C	5T	3T	5T	5T	3C	3C
I2	A & C	5A	3A	5A	5A	3C	3C
I2	C & T	5C	3C	5C	5C	3T	3T
I2	T & G	5T	3T	5T	5T	3G	3T
I2	C & T	5C	3C	5C	5C	3T	3T
E3	A,C,G	4A	1A	4A	5A	2G,1C	3G
E3	G & -	5-	1-	4-	5-	1-,2G	3G
I3	G & C	5C	1C	4C	4C	2G,1C	3 G
I3	A & G	4A,1G	1A	4A	5A	2A,1G	3A
I3	T & C	5T	1T	4T	5T	3T	2T,1C
I3	A & G	5G	1G	4G	5G	3G	2G,1A
I3	A & G	5A	1A	4A	5A	3A	2A,1G
I3	A & C	5A	1A	4A	5A	3A	2A,1C
I3	A & G	5A	1A	4A	5A	3G	3G
I3	G & A	5G	1G	4G	5G	3A	3A
I3	T & G	5T	1T	4T	5T	3G	3G
I3	G & A	5G	1A	4A	5A	3A	3A
I3	A & G	5A	1A	4A	5A	2A,1G	3A
I3	G & A	5G	1G	4G	5G	3A	3G
I3	G & A	5G	1G	4G	5G	3A	3A
I3	C & A	5C	1A	4A	5A	3A	3A
I3	G & T	5G	1G	4G	5G	3T	3T
I3	G & A	5A	1A	4A	5A	2A,1G	3A
I3	A & G	5A	1A	4A	5A	3A	2A,1G
I3	T & G	5T	1T	4T	5T	3G	3G
I3	G & C	5G	1G	4G	5G	3C	3C
I3	G & A	5A	1A	3A	5A	3G	3G
I3	C & T	5C	1C	3C	5C	3T	3T
I3	C & T	5C	1C	4C	5C	3T	3T
I3	T & G	5T	1T	4T	5T	3G	3G
I3	C & G	5C	1C	4C	5C	3G	3G
I3	C & G	5C	1C	4C	5C	3G	3G

I3	G & A	5A	1G	4A	5A	3A	3A
I3	A & G	5A	1A	4A	5A	3A	2A,1G
I3	T & G	5T	1T	4T	5T	3G	3G
I3	T & G	5T	1G	4G	5G	3G	3G
I3	A & G	5A	1A	4A	5A	3G	3G
E4	A & G	5A	1A	4A	5A	3G	3G
E4	T & C	5T	1T	4T	5T	3C	3C
E4	T & G	5T	1T	4T	5T	3G	3G
E4	C & G	5C	1C	4C	5C	3G	3G
E4	C & T	5C	1T	4C	5C	3C	3C
E4	G & A	5G	1G	4G	5G	3G	2G,1A

Figure 1. Representation of *igf2* of *G. multiradiatus* and the binding site of primers that were used for sequencing. Blue boxes represent the exons and continuous lines the introns. Green arrowheads show the binding site of the primers used for the amplification of the ≈ 5-kb fragment. Purple arrowheads show the binding site of the internal primers used for sequencing *igf2.*

Figure 2. Scheme of *igf2* of Goodeins. a) *Igf2* is composed of 4 exons and 3 introns. E denotes exons and I introns b) *igf2* of *G. multiradiatus*- Z population, c) *igf2* of *G. multiradiatus*- M population, d) *igf2* of *G. viviparous*, e) *igf2* of *A. diazi*. Numbers indicate the length (in base pairs) of every exonic or intronic region. The 17 nucleotides marked at the end of exon 4 are the 17 nucleotides we lack from the sequence of the four Goodeins.

Figure 3. Amino acid sequence of *igf2* of *G. multiradiatus* (Zempoala and San Matías el Grande populations), *G. viviparus*, *A. diazi, I. furcidens, H. formosa and Danio rerio* (copy b). X denotes a non-determined amino acid because of an incomplete triplet.

Figure 4. Phylogenetic tree of the amino acid sequence of *igf2* of *G. multiradiatus* (Zempoala and San Matías el Grande populations), *G. viviparus*, *A. diazi, I. furcidens, H. formosa and Danio rerio*. The evolution of two amino acids is mapped within the phylogeny. Numbers denotes the position within the alignment after curation, and letters the amino acid name. P= Proline, S= Serine, K= Lysine, N= Asparagine. Numbers in red indicate the branch support values.

Figure 1.

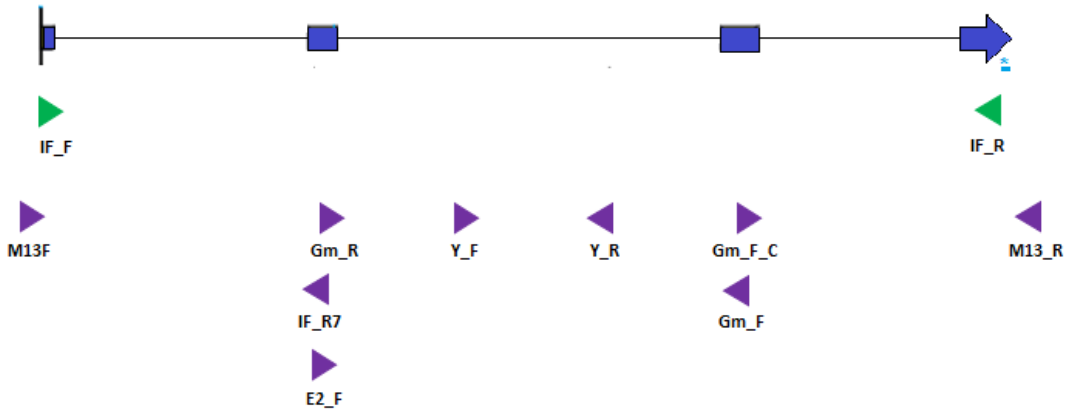


Figure 2.

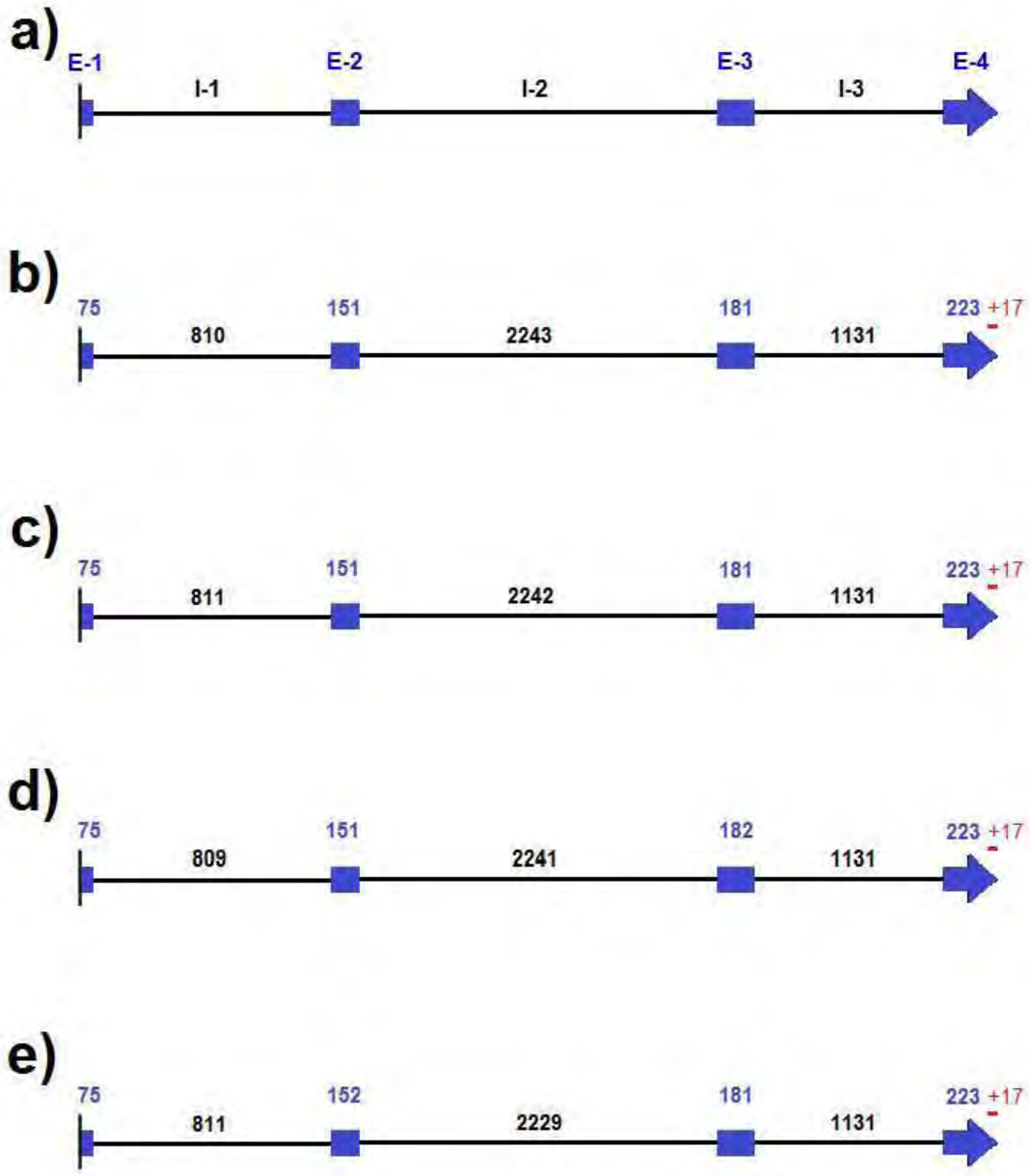

a)
E-1
E-2
E-3
E-4
I-1
I-2
I-3
b)
75
151
181
223 +17
810
2243
1131
c)
75
151
181
223 +17
811
2242
1131
d)
75
151
182
223 +17
809
2241
1131
e)
75
152
181
223 +17
811
2229
1131

Figure 3

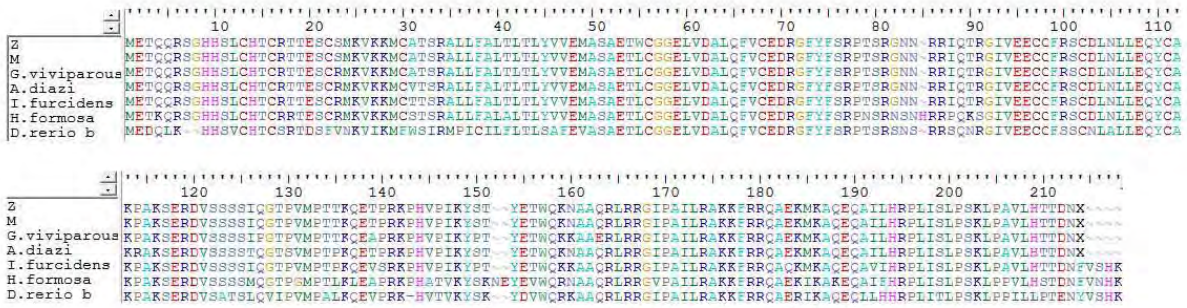
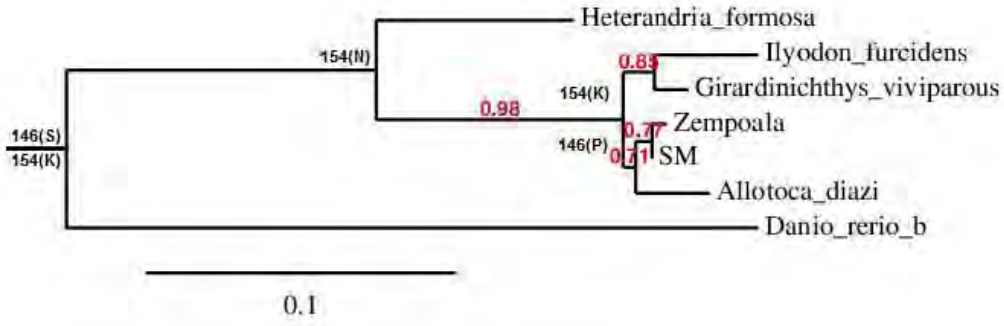

10 20 30 40 50 60 70 80 90 100 110
Z METQQRSGHHSLCHTCRTTESCSMKVKKMCATSRALLFALTLTLYVVEMASAETWCGGELVDALQFVCEDRGFYFSRPTSRGNN-RRIQTRGIVEECCFRSCDLNLLEQYCA
M METQQRSGHHSLCHTCRTTESCSMKVKKMCATSRALLFALTLTLYVVEMASAETLCGGELVDALQFVCEDRGFYFSRPTSRGNN-RRIQTRGIVEECCFRSCDLNLLEQYCA
G.viviparous METQQRSGHHSLCHTCRTTESCRMKVKKMCATSRALLFALTLTLYVVEMASAETLCGGELVDALQFVCEDRGFYFSRPTSRGNN-RRIQTRGIVEECCFRSCDLNLLEQYCA
A.diazi METQQRSGHHSLCHTCRTTESCRMKVKKMCVTSRALLFALTLTLYVVEMASAETLCGGELVDALQFVCEDRGFYFSRPTSRGNN-RRIQTRGIVEECCFRSCDLNLLEQYCA
I.furcidens METQQRSGHHSLCHTCRTTESCRMKVKKMCTTSRALLFALTLTLYVVEMASAETLCGGELVDALQFVCEDRGFYFSRPTSRGNN-RRIQTRGIVEECCFRSCDLNLLEQYCA
H.formosa METKQRSGHHSLCHTCRRTESCRMKVKKMCSTSRALLFALALTLYVVEMASAETLCGGELVDALQFVCEDRGFYFSRPNSRNSNHRRPQKSGIVEECCFRSCDLNLLEQYCA
D.rerio b MEDQLK--HHSVCHTCSRTDSFVNKVIKMFWSIRMPICILFLTLSAFEVASAETLCGGELVDALQFVCEDRGFYFSRPTSRSNS-RRSQNRGIVEECCFSSCNLALLEQYCA
120 130 140 150 160 170 180 190 200 210
Z KPAKSERDVSSSSIQGTPVMPTTKQETPRKPHVPIKYST--YETWQKNAAQRLRRGIPAILRAKKFRRQAEKMKAQEQAILHRPLISLPSKLPAVLHTTDNX----
M KPAKSERDVSSSSIQGTPVMPTTKQETPRKPHVPIKYST--YETWQKNAAQRLRRGIPAILRAKKFRRQAEKMKAQEQAILHRPLISLPSKLPAVLHTTDNX----
G.viviparous KPAKSERDVSSSSIPGTPVMPTTKQEAPRKPHVPIKYPT--YETWQKKAAERLRRGIPAILRAKKFRRQAEKMKAQEQAILHRPLISLPSKLPAVLHTTDNX----
A.diazi KRAKSERDVSSSSTQGTSVMPTPKQETPRKPHVPIKYST--YETWQKNAAQRLRRGIPAILRAKKFRRQAEKMKAQEQAILHRPLISLPSKLPAVLHTTDNX----
I.furcidens KPAKSERDVSSSSIQGTPVMPTPKQEVSRKPHVPIKYPT--YETWQKKAAQRLRRGIPAILRAKKFRRQAQKMKAQEQAVIHRPLISLPSKLPAVLHTTDNFVSHK
H.formosa KPAKSERDVSSSSMQGTPGMPTLKLEAPRKPHATVKYSKNEYEVWQRNAAQRLRRGVPAILRAKKFRRQAEKIKAKEQAIFHRPLISLPSKLPFVLHSTDNFVNHK
D.rerio b KPAKSERDVSATSLQVIPVMPALKQEVPRK-HVTVKYSK--YDVWQRKAAQRLRRGIPAILRAKKFRRQAERIKAQEQLLHHRPLITLPSKLPPILLPTENYVSHK

Figure 4


Heterandria_formosa
154(N)
Ilyodon_furcidens
0.85
154(K)
Girardinichthys_viviparous
0.98
Zempoala
146(S)
154(K)
146(P)
SM
Allotoca_diazi
Danio_rerio_b
0.1

CHAPTER 6

Males of *G. multiradiatus* seem to influence female allocation of resources (see chapter 4). IGF2, an essential protein for a proper maternal-foetal feeding communication during embryonic development is monoallelically expressed in mammals, which appears to be an outcome of a conflict between padumnal and madumnal alleles over maternal allocation of resources in placental mammals. Therian mammals share several traits with placental fish. Futhermore, *igf2* is under positive selection in placental fish as a result of parent-offspring conflict, and the evolution of IGF2 aminoacid sequence seems to be at least partialy influenced by the intensity of sexual selection within the Goodeids. Thus, could have genomic imprinting of *igf2* evolved in Goodeids and thus explain the phenotypic data previously obtained?

¿Igf2 se expresa de una manera dependiente del origen parental en un pez vivíparo matrotrófico?

Is *igf2* expressed in a parent-of-origin manner in a viviparous matrotrophic fish?

Yolitzi Saldivar Lemus[1], Jean-Philippe Vielle-Calzada[2], Michael G. Ritchie[3] & Constantino Macías Garcia[1*]

[1]Instituto de Ecología, Universidad Nacional Autónoma de México, [2] UGA Laboratorio Nacional de Genómica para la Biodiversidad CINVESTAV Irapuato México, [3]School of Biology, University of St. Andrews.

Manuscript

This manuscript is a more detailed analysis and description of molecular data publised in the article of Appendix 5

Abstract. In Therian mammals, conflict between paternal and maternal alleles is the most accepted explanation for the evolution of genomic imprinting of genes related to maternal allocation of resources during embryonic development. The evolution of placentation, which allowed a more efficient maternal-foetal physiological communication, in polygynous mating systems, however, is not exclusive of this group of vertebrates. These attributes that exacerbate the intensity of conflict between parental alleles are present in other viviparous taxa in which, therefore, it should be expected the evolution of a parent-of-origin expression of genes related to maternal allocation. We used the matrotrophic fish *Girardinichthys multiradiatus* to determine whether the insulin-like growth factor 2 –*igf2*- gene is imprinted in placental fish. We found evidence suggestive of monoallelic expression of *igf2* (only the paternal allele was transcribed), and clear evidence of a parent-of-origin methylation pattern (the maternally inherited *igf2* allele contains many more methylated 5'cytosines than the paternally inherited allele). Our sample size was very limited, which rendered out results on allelic expression inconclusive; nonetheless, our findings suggest that parental manipulation of maternal resources via *igf2* expression has evolved in fish, while the parent-of-origin methylation pattern appears to be a potential candidate mechanism modulating this antagonistic coevolution process.

Keywords. Goodeidae, genomic imprinting, DNA methylation, *Girardinichthys multiradiatus*, maternal allocation

Introduction

The interests of individuals rarely coincide in nature as they don't share the same genome. Whenever the interests of individuals (or alleles within an individual) differ, conflict is expected to arise (Parker 1979). Conflict can be related to current or future mating decisions or to how much each individual should invest in progeny (Trivers 1972).

Even if offspring are cared for by members of one sex (usually the females), manipulation may still occur if females are induced to invest preferentially in the brood of the current male, either through sensory manipulation (e.g. Burley 1986), or by enhancing the ability of the offspring to extract resources from the mother. In viviparous species, manipulation of maternal resources during development can lead females to invest more than what it is optimum, for them and for a proper embryonic development. Such manipulation can be regulated at a genetic level if parental genes expressed in the embryo (or in the placenta) are in conflict and are able to increase the offspring demand (or the placental supply) of nutrients.

Haig and Westoby (1989) suggested that, as a result of conflict between parental alleles in the developing embryo, when a loci encodes a protein responsible for resources acquisition from the mother, parent-specific gene expression would be expected to arise, where the paternally derived allele would be over-expressed and the maternally derived allele under-expressed. This parent-of-origin gene expression, known now as genomic imprinting, appears to be the evolutionary result of an antagonistic coevolution between the parental alleles (padumnal alleles -when paternally inherited- and madumnal alleles -when maternally inherited-; Wilkins & Haig 2002) in the offspring genome (The kinship Theory ;Haig 2000).

Placentas, an organ that connects the developing embryo to maternal tissue, allow a more effective nutrient provisioning and waste transfer during development. This is achieved by an efficient maternal-foetal physiological communication mediated by

hormones (Newbern & Freemark 2011). Under a Kinship theory perspective, placental hormones are interpreted as foetal attempts to manipulate maternal metabolism for foetal benefit, and once genomic imprinting evolved, it predicts that the paternally-derived allele will be active and the maternally-derived allele will be silent (Haig 1996). There is evidence that most imprinted genes are expressed in the placenta (Kaneko-Ishino et al. 2003) and that placental proteins are rapidly evolving probably as a result of the evolutionary conflict that targets the mammalian placental proteins that mediate mother-embryo interactions (Chuong et al. 2010).

Among these proteins, insulin-like growth factor 2 (IGF2) and insulin-like growth factor 2 receptor (IGF2R) have been widely studied. IGF2 regulates the nutrient demand by the foetus and the supply of maternal nutrients by the placenta (Constância et al. 2002),while IGF2R , also known as mannose-6-phosphate receptor, a monomeric transmembrane protein (Duan et al. 2010), binds and targets IGF2 (and mannose-6-phospate) for lysosomal degradation (Kornfeld 1992). The genes that encode these proteins with antagonistic effects are reciprocally imprinted in Therian mammals. *Igf2* in paternally expressed and maternally silent (DeChiara et al. 1991) and *igf2*r is maternally active and paternally silent in most marsupials and eutherian (excluding Scandentia, Dermoptera and Primates; Barlow et al. 1991; J Keith Killian et al. 2001).

Genomic imprinting of *igf2,* a gene that can influence the distribution of maternal resources, evolved, as predicted by Wilkins & Haig (2003), in viviparous placental species where females have offspring by more than one male. The function of the mannose-6-phosphate receptor of capturing IGF2 for ulterior degradation probably evolved as a response to high levels of paternal IGF2. Its imprinted status, probably followed afterwards as the outcome of selection favouring the inactivation of the parental allele (Haig & Graham 1991).

Igf2 is imprinted in eutherians and marsupials, but not in monotremes or birds (DeChiara et al. 1991; J. Keith Killian et al. 2001; Nolan et al. 2001). Apart from mammals, viviparity has evolved in several taxa with polyginous mating systems, such as fish, amphibian, resptiles and some invertebrates (Blackburn 1999).

Matrotrophy, a type of embryonic nourishment in which nutrients are supplied by any maternal mean other than yolk through gestation, has evolved in many groups of vertebrates (see Blackburn 2015), and among fishes is present in at least 11 families, where it seems to have evolved independently (Wourms et al. 1988).

The Mexican Goodeidae (Goodeinae; Lombardi & Wourms 1985a; Lombardi & Wourms 1985b), is a clade of ca. 40 species distributed in 17 or 18 genera (Webb et al. 2004), Within this family extreme sexual asymmetry in parental care that viviparity entails is characteristic (Macías Garcia 2014). Such asymmetry in parental investment is particularly large in the Goodeinae, the viviparous subfamily within the goodeids. Goodein females nourish their embryos through the trophotaenial placenta, a placenta-like structure where specialized embryonic tissues are in apposition with the ovarian lumen (Schindler 2005; Uribe & García Alarcón 2005; Lombardi & Wourms 1985a; Lombardi & Wourms 1985b), for 7-8 weeks (Macías-Garcia & Saborío 2004), during which embryos can grow up to 15000% (Lombardi & Wourms 1988). Furthermore, *igf2* is present in placental fishes, where parent-offspring conflict has being driving its evolution (O'Neill et al. 2007), and it is transcribed in embryos (YSL unpublish data).

Extended maternal provisioning and the evolution of the trophotaenial placenta makes Goodeinae fish potentially good models for the study of the evolution of genomic imprinting of *igf2* as a consequence of conflict between padumnal and madumnal alleles. We used fish from several propulations of The Amarillo *(Girardinichthys multiradiatus)* to determine if genomic imprinting of *igf2* has evolved as a response to selective pressures acting on the parental alleles.

Methods

Study species

Girardinichthys multiradiatus is a goodein fish endemic from Central Mexico. It is found in water bodies of the upper Lerma River basin, and in limited regions of the Balsas and Pánuco catchments (Gesundheit & Macias-Garcia 2005). Males have much larger and colourful dorsal fins than females and males also must perform complex courting behaviours to gain female's acceptance for mating (Macias-Garcia et al. 1994; Macías Garcia et al. 1998; González Zuarth & Macías Garcia 2006). There has been rapid population divergence (Ritchie et al. 2007; Macías Garcia et al. 2012), and female mate choice frequently leads to pre-mating isolation between populations (González Zuarth & Macías Garcia 2006; Macías Garcia et al. 2012).

We used fish from the Zempoala –Z- population (in the Zempoala lakes National Park, Estado de México), from Huapango –H- population (Michoacan state), and from Tonatiahua –T- population (also in the Zempoala lakes National Park).

Fish were collected under SAGARPA permit DGOPA/01262/040310.0716 and were promptly transported to aquaria at the Instituto de Ecología, UNAM in local water, Stress Coat and antiseptics. They were kept under standardised conditions: a 12-hour-day-night cycle, 21ºC and they fed SeraVipan™ commercial fish flakes twice a day.

All methods were carried out in accordance with the Guidelines for the treatment of animals in behavioural research and teaching published by Animal Behaviour (DOI:10.1016/j.anbehav.2011.10.031). Fish were kept at the Instituto de Ecología, UNAM.

Parent of origin *igf2* expression

Genotyping of Families

To evaluate the expression pattern of *igf2*, we generated several breeding groups (2 or 3 fish), always made of one Z-Z pair, and in some cases an additional female from either

Huapango or Tonatiahua. Fish within an inter-population pair were raised together to overcome preferences for intra-population partners (De Gasperin & Macías Garcia 2014). Each pregnant female and her entire brood were sacrificed around the 7th week of pregnancy. After we processed all pregnant females from each breeding group, we collected a fin clipping from the sire. All the tissues were stored either in absolute ethanol or in RNAlater.

Exon 2 seemed to be the most polymorphic one (see results from chapter 5), thus we screened for SNPs from exon 2 of 22 breeding pairs (36 individuals, as five males were shared by two females and one male by 3 females). Primers (Table1; Figure 1) amplified a product of 443 nucleotides that contained exon 2 in its entirety, plus some segments of introns 1 and 2.

Genomic DNA was extracted with a homemade protocol (see supplementary material for details). The PCR reaction system contained 10 µl of GoTaq Green Master Mix (Promega), 6 µl Milli Q water, 1 µl DMSO, 1 µl I2_F_P (20 pmol/µl), 1 µl I2_R_P (20 pmol/µl), 1 µl DNA, and the PCR amplification conditions were: 30 PCR cycles of 95 ºC for 5 min, 94 ºC for 30 s, 59 ºC for 30 s, 72 ºC for 30 s followed by 10 min at 72 ºC. PCR products were cloned using TOPO TA Cloning kit (Invitrogen) for electrocompetent cells (TOP10 Electrocomp). Plasmid DNA was extracted according to the alkaline lysis protocol by Sambrook and co-workers (1989) with some minor modifications. A minimum of 10 clones per fish were sent for sequencing, and sequences were analysed with BioEdit Sequence Alignment Editor. We genotyped the offspring of families 4 and 21 (Table 2), in which we could track the parental alleles (i.e. parents were not homozygous for the same SNP), and screened the brood for heterozygous embryos, as before.

Assessing gene expression through RT-PCR

It has been demonstrated that *IGF2* synthesis is higher in the early stages of embryonic development in *Heterandria formosa* embryos (Schrader & Travis 2012); however, we used

near-terminus embryos for the assessment of allelic expression since early pregnancy is undetectable, thus we had to wait until it was evident rather than unnecessarily sacrificing many females.

Total mRNA of heterozygous offspring was isolated using TRIzol (Invitrogen) and was then reverse-transcribed using SuperScript II Reverse Transcriptase and oligodT primers, according to the manufacturer's protocol (Invitrogen). The cDNA was employed as a template for PCR amplification using specific primers. Primers (Table 1) were designed to amplify exon 1 and 2 in order to do distinguish the size of the amplified cDNA product from genomic fragments that could be amplified after inefficient DNAse digestion (Figure 1). We cloned the fragment as before, analysed a minimum of 50 clones per embryo and determined which allelic variant (parental allele) had been recovered.

Bisulfite sequencing

Parent of origin expression effects often occur by genomic DNA methylation, involving the addition of a methyl group to cytosine residues of the dinucleotide CpG (Hendrich & Tweedie 2003). To determine whether the asymmetric effects on offspring size could be influenced by parent of origin effects in the methylation state of *igf2*, we took advantage of a heterozygous C/T embryo (P21-3) that inherited a T allele from its mother and a C allele from its father, and of a heterozygous C/T adult female (P11-F) -although here we did not know the parental origin of each allele- and analysed the pattern of 5'cytosine methylation in a 443 bp fragment that spanned the SNP site by treating genomic DNA with bisulfite before PCR amplification and cloning.

Bisulfite sequencing was performed as reported in Lim et al. (2015) with minor modifications using DNA from two individuals: a heterozygous adult female ((P11-F; Table 2) and a heterozygous embryo (P21-3; table 2). Samples of 500 ng of genomic DNA (obtained as above; see supplementary material for details) were bisulfite converted using

EZ DNA methylation-direct kit (Zymo Research), eluted in 30 µl elution buffer, and 1 µl of each aliquot was PCR amplified using primers forward: Forward1 and Forward2 and reverse: Reverse1 and Reverse2 (designed as above; see appendix, Table S2) (95 ºC for 5 min, 20 cycles of 94 ºC for 30 s, 59 ºC for 30 s, 72 ºC for 30 s each, 72ºC for 10 min.). PCR products were gel purified, cloned into pDRIVE cloning vector using Qiagen PCR cloning kit (Qiagen, Valencia, CA) and transformed into DH10B cells before sequencing.

Results

Parent of origin *igf2* expression

We found only one SNP (C/T) sufficiently frequent to be used as a marker of parent-of-origin expression of *igf2*. Individuals were either homozygous for T (T/T) or heterozygous T/C; no homozygous C/C individuals were recovered (Table 3) for the informative SNP. As the frequency of C was very low (0.047) this was within HW expectations.

To determine the genotypes of *igf2* transcripts present in developing embryos, we conducted RT-PCR of the *igf2* gene by amplifying a 201 bp cDNA fragment spanning the SNP. The cross analysed was between a homozygous T/T male and a heterozygous C/T female, so half the offspring should have been heterozygous. The family included two heterozygous offspring out of six. *Igf2* RT-PCR amplification yielded 78 and 54 independent cDNA fragments from these two individuals that were sequenced after cloning. The sequence of all recovered cDNAs corresponded to only the paternally inherited allele, suggesting that the maternal allele is not expressed in the developing embryos (Table 3).

Bisulfite sequencing

Thirty-eight independent fragments of *igf2* from P21-3 were sequenced: 20 corresponded to the maternally and 18 to the paternally inherited *igf2* copy ($X^2 = 0.105$, $df = 1$, $p > 0.05$). Strikingly, 5'-methylcytosines in a CpG context were only prevalent (i.e. present

in > 50% of the clones) in sequences representing a maternally inherited *igf2* copy, and were virtually absent from copies that were paternally inherited (Figure 2a). Additional cytosines present in non-CpG positions were also frequently methylated in the maternally inherited *igf2* copy, contributing to a highly contrasting methylation pattern that correlates with the mono-allelic expression of *igf2* during embryogenesis.

Seventeen independent sequences from adult female P11-F were obtained; the eight belonging to one allele were hypomethylated, and the nine sequences of the other allele were hypermethylated (Figure 2b). As with P21-3, these segregations are not different from 1:1 ($X^2 = 0.06$, $df = 1$, $p > 0.05$), indicating that the cytosine residue present at position 225 of the amplified fragment (corresponding to the P21-3 paternally inherited *igf2* copy) is not affected by the bisulfite treatment, allowing for a comparison of the methylation pattern among both alleles.

Discussion

The *igf2* gene encodes insulin-like growth factor 2 (IGF2) which plays an important role in embryonic development. It is involved in nutrient exchange between mother and embryo (Constância et al. 2002; Reik et al. 2003), and can, therefore, affect the amount of nutrients transferred to the developing offspring. An analysis of nonsynonymous mutations within the coding sequence of *igf2*, demonstrated that this gene is under positive selection in several placental cyprinodontifomes (O'Neill et al. 2007), which implies that *igf2* plays a role during embryonic development of viviparous fish. O'Neill and co-workers (2007) argued that they found evidence of sustained directional selection on the coding sequence of this gene in matrotrophic cyprinodontiformes that suggests that a parent-offspring conflict is driving *igf2* evolution. This is plausible, but the argument cannot be compelling unless it is also shown that either 1) an antagonistic gene (e.g. the *igf2r*) has experienced a comparable evolutionary divergence, or 2) that the expression of *igf2* in the embryos follows a parent-of-

origin pattern (i.e. that there is a bias in embryos to express the paternal allele). Although suggestive, our assessment of parent of origin expression of *igf2* is inconclusive given the scarcity of heterozygous fish. We only demonstrated a parent-of-origin methylation pattern in the developing embryos, but these data, together with 1) the evidence of *igf2* being expressed in fish embryos (Yuan et al. 2011; Lawton et al. 2005), 2) a parent-specific methylation pattern in gametes of an oviparous fish (suggesting that the foundations of genomic imprinting also exist in teleost fish (Xie et al. 2009), and 3) the evolution in fish of the manose-6-phosphate receptor into an insulin-like growth factor 2 receptor (*igf2r*) with a role on *igf2* degradation (Nolan et al. 2006) that has a similar structure and affinity for IGF2 to that of the mammalian gene (Méndez et al. 2001), suggests that the possibility of the evolution of genomic imprinting in this group of viviparous vertebrates should be investigated.

Our evidence of monoallelic embryonic expression of *igf2* derives from the finding of a C-T SNP at site 44 of exon 2, with the C version of this SNP recovered in a very low proportion. Since taq polymerase may produce transitions from T to C (one of the most common mutations induced by taq polymerases58), it is conceivable that the individuals that we genotyped as heterozygous for this SNP were in fact errors due to defective taq activity. We confirmed that this was not the case, because the bisulfite sequencing of genomic DNA from those individuals yielded an abundance of 5'-methylcytosines and of thymine in the expected proportion of roughly 50-50 %. Yet, highly methylated sequences (or alleles) tend to amplify at a much lower efficiency during PCR than poorly or non-methylated ones (Wojdacz et al. 2009), which may also explain the low proportion of the C allele in the genotyping. Furthermore, we confirmed that this SNP in Zempoala population is at Hardy-Weinberg equilibrium.

The sexually-antagonistic IGF system is only known to occur in mammals, but its constitutive elements are found in fish, raising the possibility that it evolved independently in

mammals and teleosts, or that it was present in the ancestors of the two lineages diverged. Previous efforts to demonstrate imprinting of *igf2* in placental poeciliid species have been unsuccessful (Lawton et al. 2005) yet we found evidence that suggest parent-of-origin gene expression in the Goodeidae (which are also cyprinodontids). Some attributes that may favour the evolution of a genetic antagonistic coevolution mediated by IGF2 in the Goodeidae include enforceable female mate choice. This may be linked to the fact that goodeid embryos' dry weight can increase up to 15000% (*A*meca *splendens;* Lombardi & Wourms 1988), whereas placental poeciliids embryos achieve a more modest or 1800% (*Poeciliopsis turneri*), to 4000% (*H. formosa*; Wourms 1981). Such greater mass increase takes place during a gestation period that lasts about eight weeks; twice as much that of poeciliids. We think that the massive reproductive allocation of goodeid females, together with the existence of a trophotaenial placenta (a foetal structure involved in the capture and transport of nutrients from the ovarian lumen/walls to the embryonic gut; Lombardi & Wourms 1985a; Lombardi & Wourms 1985b) provide both the opportunity and the physiological conditions in which *igf2* can influence maternal investment.

Our results are consistent with expectations derived from sexual conflict (Parker 1979; Parker 2006). Goodeid matrotrophic viviparity involves a massive, protracted transfer of nutrients to the embryos (Lombardi & Wourms 1985a; Lombardi & Wourms 1985b) that can be co-opted by males. There is no evidence suggesting that males can influence female investment through sensory stimulation during courtship, but we show evidence that *igf2*, a gene whose overexpression may influence embryonic growth, has a parent-specific methylation pattern, which suggest a possibly epigenetic parental effect, as such described in mammals (Murrell et al. 2004; Lawton et al. 2008). Furthermore, *igf2* pruduction is tissue-specific and the methylation pattern is stage-specific (Kafri et al. 1992). We used the whole body of embryos of the relatively the same age to look at gene expression and methylation

pattern, thus, more research should be address to have a better understanding of the epigenetic regulation of *igf2* in goodeids.

The breeding system of the Amarillo (*Girardinichthys multiradiatus*), fits the conditions stipulated by Wilkins and Haig(Wilkins & Haig 2003) as potential promoters of genomic imprinting; 1) broods can be sired by more than one male (Macías-Garcia & Saborío 2004), 2) females bear the bulk of the reproductive costs (e.g. Lombardi & Wourms 1985a; Lombardi & Wourms 1985b), and 3) their allocation of resources can be influenced by genes that are expressed in the embryos (e.g. *igf2*; see O'Neill et al. 2007); therefore, further research on the possibility of genomic imprinting of *igf2* of this fish is needed.

Acknowledgments

We thank T. Sneddon, J. Graves, G. Smith and C. Álvarez for technical support. Edgar Ávila-Luna and Marcela Méndez Janovitz helped with the fish husbandry. Research was supported by PAPIIT grant IN216111 (CMG), CONACyT PhD scholarship 351769 (YSL) and the Howard Hughes International Research Program (JPVC). This work was conducted as partial fulfilment of YSL PhD thesis within the Doctorado en Ciencias Biomédicas PhD program (UNAM) under the supervision of CMG.

References

Barlow, D.P. et al., 1991. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature*, 349, pp.84–87.

Bird, A., 2002. DNA methylation patterns and epigenetic memory DNA methylation patterns and epigenetic memory. *Genes & Development*, 16, pp.6–21.

Blackburn, D.G., 2015. Evolution of vertebrate viviparity and specializations for fetal nutrition: A quantitative and qualitative analysis. *Journal of Morphology*, 276(8), pp.961–990.

Blackburn, D.G., 1999. Viviparity and oviparity: Evolution and reproductive strategies. *Encyclopedia of Reproduction*, 4, pp.994–1003.

Burley, N., 1986. Comparison of the band-colour preferences of two species of estrildid finches. *Animal Behaviour*, 34(6), pp.1732–1741.

Chuong, E.B., Tong, W. & Hoekstra, H.E., 2010. Maternal-Fetal Conflict: Rapidly Evolving Proteins in the Rodent Placenta. *Molecular Biology and Evolution*, 27(6), pp.1221–1225.

Constância, M. et al., 2002. Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature*, 417(6892), pp.945–948.

DeChiara, T.M., Robertson, E.J. & Efstratiadis, A., 1991. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell*, 64(4), pp.849–859.

Duan, C., Ren, H. & Gao, S., 2010. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: Roles in skeletal muscle growth and differentiation. *General and Comparative Endocrinology*, 167(3), pp.344–351.

De Gasperin, O. & Macías Garcia, C., 2014. Congenital predispositions and early social experience determine the courtship patterns of males of the Amarillo fish. *Behavioral Ecology and Sociobiology*, 68(4), pp.639–648.

Gesundheit, P. & Macias-Garcia, C., 2005. Biogeografía cladística de la familia Goodeidae (Cyprinodontiformes). In J. Llorente-Bousquets & J. Morrone, eds. *Regionalización biogeográfica en Iberoamérica y tópicos afines*. Ciudad de México: Facultad de Ciencias, UNAM, pp. 319–338.

González Zuarth, C. & Macías Garcia, C., 2006. Phenotypic differentiation and pre-mating isolation between allopatric populations of Girardinichthys multiradiatus. *Proceedings. Biological sciences / The Royal Society*, 273(1584), pp.301–7.

Haig, D., 1996. Placental hormones, genomic imprinting, and maternal-fetal communication. *Journal of Evolutionary Biology*, 9(3), pp.357–380.

Haig, D., 2000. The Kinship Theory of Genomic Imprinting. *Annual Review of Ecology and Systematics*, (34), pp.9–32.

Haig, D. & Graham, C., 1991. Genomic Imprinting and the Strange Case of the Insulin-like Growth Factor II Receptor. *Cell*, 64, pp.1045–1046.

Haig, D. & Westoby, M., 1989. Parent-Specific Gene Expression and the Triploid Endosperm. *The American Naturalist*, 134(1), p.147.

Kafri, T. et al., 1992. Developmental pattern of gene-specific DNA methylation in the mouse embryo and germline. *Genes Dev.*, 6, pp.705–714.

Kaneko-Ishino, T., Kohda, T. & Ishino, F., 2003. The Regulation and Biological Significance of Genomic Imprinting in Mammals. *Journal of Biochemistry*, 133(6), pp.699–711.

Killian, J.K. et al., 2001. Divergent evolution in M6P/IGF2R imprinting from the Jurassic to the Quaternary. *Human Molecular Genetics*, 10(17), pp.1721–1728.

Killian, J.K. et al., 2001. Monotreme IGF2 expression and ancestral origin of genomic imprinting. *Journal of Experimental Zoology*, 291(2), pp.205–212.

Kornfeld, S., 1992. Structure and function of the mannose 6-phosphate insulin-like growth factor-II receptors. *Annu. Rev. Biochem*, 61, pp.307–330.

Lawton, B.R. et al., 2005. Allelic expression of IGF2 in live-bearing, matrotrophic fishes. *Development Genes and Evolution*, 215, pp.207–212.

Lawton, B.R. et al., 2008. Genomic imprinting of IGF2 in marsupials is methylation dependent. *BMC genomics*, 9, p.205.

Lombardi, J. & Wourms, J.P., 1988. Embryonic growth and trophotaenial development in goodeid fishes (Teleostei: Atheriniformes). *Journal of Morphology*, 197(2), pp.193–208.

Lombardi, J. & Wourms, J.P., 1985a. The trophotaenial placenta of a viviparous goodeid fish. I: Ultrastructure of the Internal Ovarian Epithelium, the Maternal Component. *Journal of molecular biology*, 184, pp.277–292.

Lombardi, J. & Wourms, J.P., 1985b. The trophotaenial placenta of viviparous goodeid fish. II. Ultrastructure of the trophotaenia, the embryonic component. *Journal of Morphology*, 184(3), pp.293–309.

Macias-Garcia, C., Jiménez, G. & Contreras, B., 1994. Correlational evidence of a sexually-selected handicap. *Behavioral Ecology and Sociobiology*, 35, pp.253–259.

Macías-Garcia, C. & Saborío, E., 2004. Sperm competition in a viviparous fish. *Environmental Biology of Fishes*, 70(3), pp.211–217.

Macías Garcia, C., 2014. Mode of Reproduction, Mate Choice, and Species Richness in Goodeid Fish. In R. H. Macedo & G. Machado, eds. *Sexual Selection: Perspectives and Models from the Neotropics*. San Diego: Academic Press, pp. 253–288.

Macías Garcia, C. et al., 2012. Variation in sexual dimorphism and assortative mating do not predict genetic divergence in the sexually dimorphic Goodeid fish Girardinichthys multiradiatus. *Current Zoology*, 58(3), pp.440–452.

Macías Garcia, C., Saborío, E. & Berea, C., 1998. Does male-biased predation lead to male scarcity in viviparous fish? *Journal of Fish Biology*, 53(supl. A), pp.104–117.

Méndez, E. et al., 2001. Identification of a type II insulin-like growth factor receptor in fish embryos. *Endocrinology*, 142(3), pp.1090–1097.

Murrell, A., Heeson, S. & Reik, W., 2004. Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. *Nature genetics*, 36(8), pp.889–893.

Newbern, D. & Freemark, M., 2011. Placental hormones and the control of maternal metabolism and fetal growth. *Current opinion in endocrinology, diabetes, and obesity*, 18, pp.409–16.

Nolan, C.M. et al., 2001. Imprint status of M6P/IGF2R and IGF2 in chickens. *Development Genes and Evolution*, 211(4), pp.179–183.

Nolan, C.M. et al., 2006. Mannose 6-phosphate receptors in an ancient vertebrate, zebrafish. *Development Genes and Evolution*, 216, pp.144–151.

O'Neill, M.J. et al., 2007. Ancient and continuing Darwinian selection on insulin-like growth factor II in placental fishes. *Proceedings of the National Academy of Sciences of the*

United States of America, 104(30), pp.12404–12409.

Parker, G.A., 2006. Sexual conflict over mating and fertilization: an overview. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 361(1466), pp.235–259.

Parker, G.A., 1979. Sexual selection and sexual conflict. In M. Blum & N. Blum, eds. *Sexual Selection and Reproductive Competition in Insects*. New York: New York Academy Press, pp. 123–166.

Reik, W. et al., 2003. Regulation of supply and demand for maternal nutrients in mammals by imprinted genes. *The Journal of physiology*, 547(1), pp.35–44.

Ritchie, M.G. et al., 2007. Sex and differentiation: Population genetic divergence and sexual dimorphism in Mexican goodeid fish. *Journal of Evolutionary Biology*, 20, pp.2048–2055.

Sambrook, J., Fritsch, E.F. & Maniatis, T., 1989. *Alkaline Lysis Plasmid Mini-Prep Protocol*, New York: Cold Spring Harbor Laboratory Press.

Schindler, J., 2005. Digestion of embryotrophic protein in Goodeid teleosts. In M. C. Uribe & H. J. Grier, eds. *Viviparous fishes*. Florida: New Life Publication, pp. 266–267.

Schrader, M. & Travis, J., 2012. Embryonic IGF2 Expression Is Not Associated with Offspring Size among Populations of a Placental Fish. , 7(9), pp.1–5.

Uribe, M.C. & García Alarcón, A., 2005. The ovary of viviparous teleosts. Morphological differences between the ovaries of Goodea atripinnis and Ilydon whitei (Goodeidae). In M. C. Uribe & H. J. Grier, eds. *Viviparous fishes*. Florida: New Life Publication, pp. 217–235.

Webb, S.A. et al., 2004. Molecular phylogeny of the livebearing Goodeidae (Cyprinodontiformes). *Molecular Phylogenetics and Evolution*, 30(3), pp.527–544.

Wilkins, J.F. & Haig, D., 2002. Parental modifiers, antisense transcripts and loss of imprinting. *Proceedings. Biological sciences / The Royal Society*, 269(1502), pp.1841–1846.

Wilkins, J.F. & Haig, D., 2003. What good is genomic imprinting: the function of parent-specific gene expression. *Nature Reviews Genetics*, 4(5), pp.359–368.

Wojdacz, T.K., Borgbo, T. & Hansen, L.L., 2009. Primer design versus PCR bias in methylation independent PCR amplifications. *Epigenetics*, 4(4), pp.231–234.

Wourms, J.P., 1981. Viviparity: The maternal-fetal relationship in fishes. *Integrative and Comparative Biology*, 21(2), pp.473–515.

Wourms, J.P., Grove, B. & Lombardi, J., 1988. The maternal-embryonic relationship in viviparous fishes. In W. Hoar & D. Randall, eds. *Fish Physiology*. New York: Academic Press, pp. 1–134.

Xie, B. et al., 2009. The evolutionary foundation of genomic imprinting in lower vertebrates. *Chinese Science Bulletin*, 54(8), pp.1354–1360.

Yuan, X.-N. et al., 2011. Functional conservation and divergence of duplicated insulin-like growth factor 2 genes in grass carp (Ctenopharyngodon idellus). *Gene*, 470(2011), pp.46–52.

Table 1. Sequence of the primers used to amplify different fragments of *igf2.*

Primer	Sequence
Genotyping	
I1_F2_P	5’ GAGTTACCAGGTCAGTGCGT 3’
I2_R2_P	5’ AGAGGAAAGGGGAGCGAAAA 3’
RTPCR	
Gm_A_F	5’ GGAGACCCAGCAAAGATCCG 3’
IF_R7	5’ GATGCGCTGCAGTTTGTCTG 3’
Bisulfite sequencing	
Forward 1	5'GAGTTACCAGGTCAGTGCGT3'
Forward 2	5’GAGTTATTAGGTTAGTGTGT3’
Reverse 1	5'AGAGGAAAGGGGAGCGAAAA3’
Reverse 2	5’AGAGGAAAGGGGAGTGAAAA3’

Table 2. Analysis of the sequence of exon 2 of *igf2* of 22 families of *G. multiradiatus*. Z = Zempoala, H = Huapango T = Tonatiahua. T and Z are mountain lakes separated by less than 0.5 Km. Crosses that could have been used for the parent of origin methylation pattern are marked with an *. The cross used for the allelic expression essay is marked with ** and the cross used for the bisulfite sequencing is marked with ***.

Family	Female ID	Male ID	Number of female clones sequenced			Number of male clones sequenced			Brood size
			T	C	Total	T	C	Total	
1	1Z	1Z	9	0	9	9	0	9	NR
2*	2T	1Z	7	2	9	9	0	9	7
3	3Z	3Z	7	0	7	11	0	11	5
4**	4Z	4Z	4	1	5	10	0	10	6
5	7H	4Z	9	0	9	10	0	10	3
6	8Z	6Z	7	0	7	4	0	4	2
7	9H	7Z	8	0	8	8	0	8	5
8	10Z	8Z	10	0	10	8	0	8	9
9	14Z	8Z	9	0	9	8	0	8	10
10*	12Z	10Z	4	0	4	4	6	10	3
11	23Z	10Z	5	5	10	4	6	10	11
12*	13Z	11Z	10	0	10	9	1	10	9
13	21H	11Z	8	2	10	9	1	10	10
14	22Z	11Z	6	4	10	9	1	10	8
15	15Z	13Z	10	0	10	10	0	10	7
16	16Z	14Z	7	3	10	1	0	1	14
17*	17Z	15Z	6	4	10	7	0	7	3
18	18Z	16Z	10	0	10	5	0	5	7
19	19H	16Z	8	0	8	5	0	5	7
20	20H	17Z	5	5	10	4	7	11	11
21***	24Z	18Z	12	0	12	7	3	11	9
22	25Z	19Z	9	1	10	9	1	10	13

NR. Not recorded

Table 3. Genotyping adults and offspring for the informative SNP. Thirty six individuals were genotyped. Only five fish from Huapango and one fish from Tonatiahua were used for the crosses, the other 30 individuals being from Zempoala. We did not find any homozygous CC individuals. We genotyped 6 offspring of one C/T x C/C cross (family 4) and used two heterozygous offspring P4-1 and P4-5 for the parent of origin *igf2* expression assay.

Numbers of adults that were genotyped

T/T ♀			**T/T ♂**			**T/C ♀**			**T/C ♂**			**C/C ♀**			**C/C ♂**		
13			9			9			5			0			0		
Z	T	H	Z	T	H	Z	T	H	Z	T	H	Z	T	H	Z	T	H
10	0	3	9	0	0	6	1	2	5	0	0	0	0	0	0	0	0

Parent of origin *igf2* expression of a C/T x C/C cross

Offspring ID	**Genotyping**			**RT-PCR**		
	Number of clones			Number of clones		
	Paternal T allele	Maternal C allele	Total	Paternal T allele	Maternal C allele	Total
P4-1	64T	4C	68	78T	0C	78
P4-2	17T	0C	17			
P4-3	7T	0C	7			
P4-4	20T	0C	20			
P4-5	18T	1C	19	54T	0C	54
P4-6	20 T	0C	20			

Z= Zempoala, T=Tonatiahua, H= Huapango.

Figure 1. Scheme of *igf2* of *G. multiradiatus.* Blue boxes represent exons, continuous lines introns, and the red arrow shows the approximate location of the selected SNP. Green arrowheads represent the binding site of the primers used for genotyping, and purple arrowheads show the binding site of the primers used for RT-PCRs.

Figure 2. Parent of origin effects in genomic DNA methylation at the *igf2* gene. 5' to 3' linear representation of cytosines present in a 443 bp genomic fragment spanning an informative SNP (highlighted in yellow) that allow distinction between maternally and paternally inherited IGF2 gene copies in a) a heterozygous offspring (P21-3) and b) a heterozygous female (P11-F); 5' methylated cytosines in a CpG context are represented by dark red dots, 5' methylated cytosines in a different context are shown as light red dots, unmethylated cytosines are indicated as blue dots, and citosines of undetermined methylation status are indicated as black dots . The lineal sequence of the fragment is shown below the graphic depiction of methylation. The cytosines are highlighted in light blue, and the nucleotide of the SNP (C/T) is highlighted in yellow.

Figure 1.

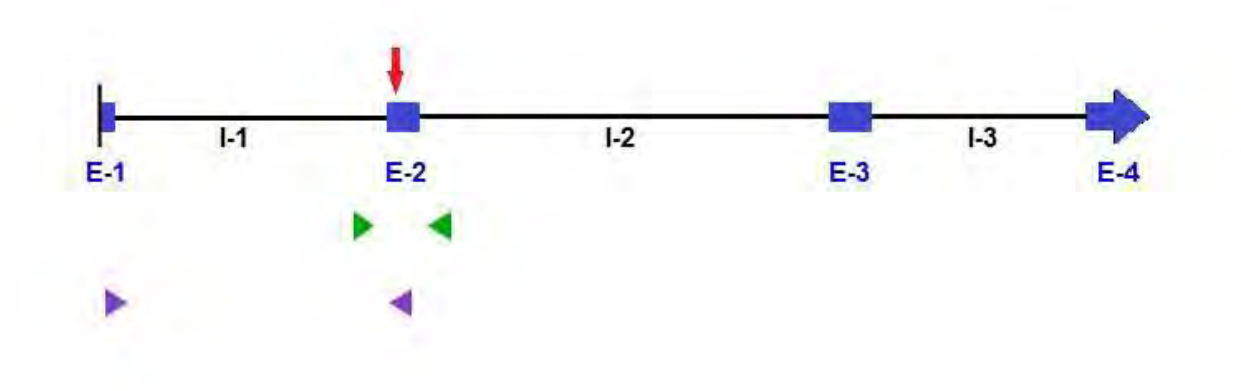
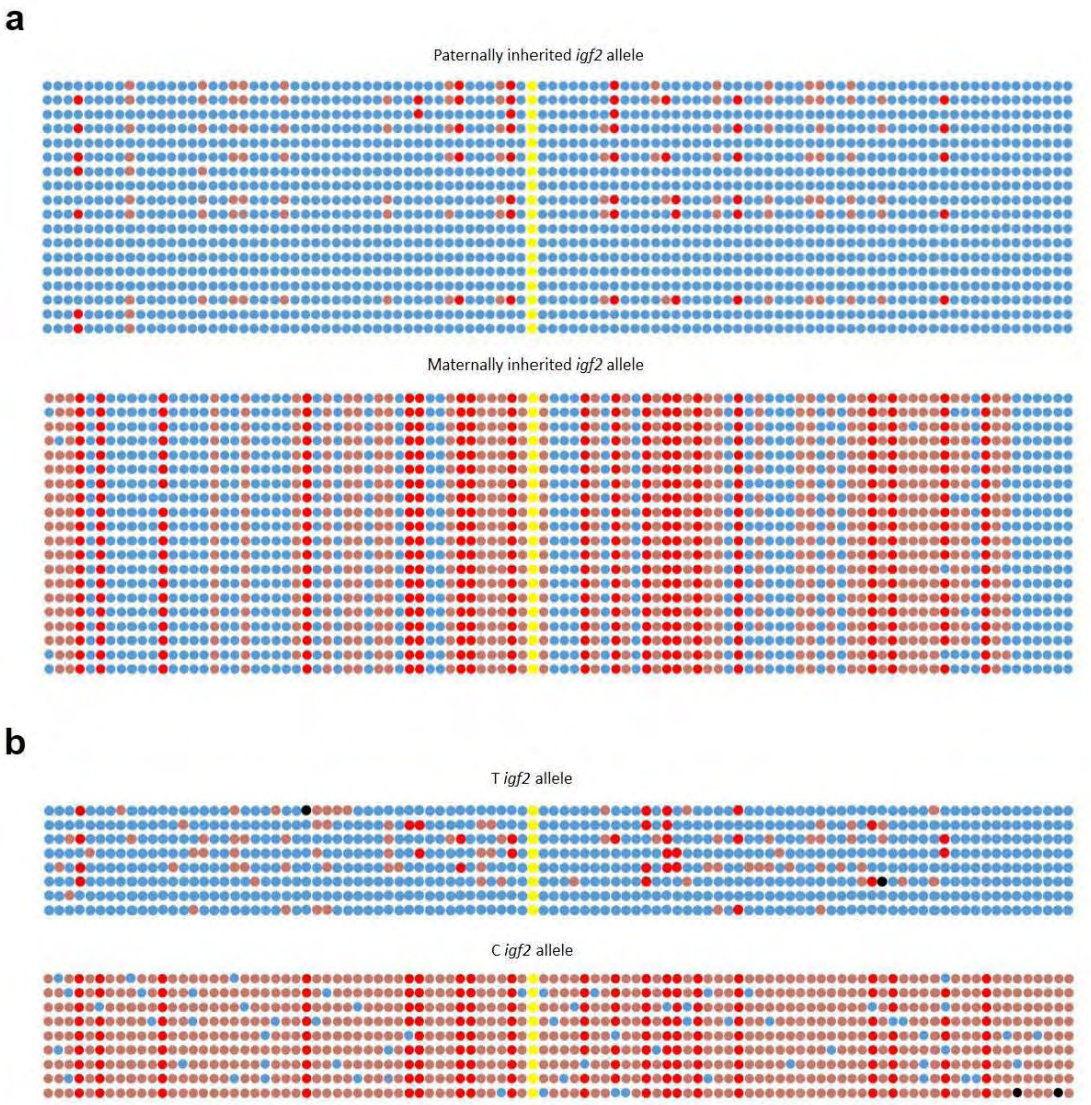


Figure 2



5'GAGTTACCAGGTCAGTGCGTGAAACAGCGTTTAAGACTTTAATCTCTTACATCTCGTACAAAACAAAACATCTGGCTATTATTTGAGT
TCTTTACATATAATTTTTATTGTCATAATAATGGATCACAAGTCTAACATTTTTCCGAATCTTATGCTTTAATTTGTGTGTTTCCCCCTGCAG
GTCAAGAAGATGTGCGCGACCAGCCGTGCGCTGCTCTTTGCGCTGACCCTCACGCTCTACGTTGTGGAAATGGCCTCGGCAGAGACG
TTGTGTGGCGGAGAGCTGGTGGATGCGCTGCAGTTTGTCTGCGAAGACAGAGGCTTCTATTTCAGTAGGTTTTTTTTTTTTCAGAGCT
ATGCAAGTTTCTCCAAAAACTAGCTGCGCAAATGTTGATTCGCCTACCTTTTTAATGTTATTCGCCCTTTTCGCTCCCCTTTCCTCT3'

Appendix

DNA extraction protocol

Cell Lysis solution

0.1M EDTA
0.2M Tris pH 8.5
1% SDS

Others:

20mg/ml Proteinase K
5 M Potassium Acetate
10mg/ml Rnase A
Isopropanol
70% Ethanol
Distilled H_20 or TE

Protocol

1. Chill a 1.5ml tube containing 600ul Cell Lysis solution on ice (may turn cloudy).
2. Add 10-20 mg of fresh or frozen tissue to the solution. Remove from ice and quickly homogenise with a microfuge tube pestle. Keep on ice.
3. Incubate at 55^0C for 15-60 minutes, for maximum yield add 3ul of Proteinase K and incubate at 55^0C overnight.
4. Add 3ul of RNase A
5. Mix by inverting tube many times at 37^0C for 15-60 mins and put them on ice.
6. Add 200ul 5M KAc.
7. Put the tubes on ice for five minutes and vortex vigorously at high speed for 20 seconds
8. Centrifuge top speed in microfuge for 5 mins at 4ºC to precipitate proteins. The precipitated proteins should form a tight pellet, if not then re-vortex for 20 seconds to mix sample and incubate on ice for 5 mins. Re- centrifuge top speed for 3 mins.
9. Decant supernatant containing DNA into a clean 1.5ml eppendorf tube containing 600ul of cold (from freezer) 100% isopropanol. Mix the sample until DNA clumps form. $1/10^{th}$ volume of 3M NaAc, pH5.2 can be added to help precipitation of DNA. DNA can be left at -80 for 1hr or overnight.
10. Centrifuge at top speed for 2 min at 4ºC.
11. Pour off supernatant and drain tube on clean absorbent paper. Add 600ul of 70% ethanol to wash pellet. Centrifuge for 1 min at 4ºC, pour off alcohol, drain then REPEAT AGAIN.
12. Centrifuge top speed in microfuge for 1 min.
13. Pipette off any residual ethanol and air dry for approx. 20 mins – don't leave drying too long or DNA becomes difficult to re suspend. (all ethanol should be gone before re suspending)
14. Re suspend in TE or H_20 (PREFFERED FOR PCR). The DNA may require heating to re suspend, heat at 65 degrees for 1 hour, tap tube periodically to aid in dispersing the DNA.
15. Store DNA samples in freezer -20 degrees long term.

CAPÍTULO 7

DISCUSIÓN GENERAL Y CONCLUSIONES

La evolución del conflicto entre individuos es ubicua en la naturaleza. Dentro de una misma especie, puede darse entre los alelos parentales en un mismo individuo, entre hermanos dentro de una misma camada, entre padres y críos, o entre parejas reproductivas (Zeh & Zeh 2008).

El conflicto relacionado a la asignación parental de recursos siempre ha existido. Para los críos siempre será benéfico que sus padres inviertan más recursos o más cuidado parental en ellos. Desde el punto de vista del conflicto sexual, para los miembros de un sexo siempre será beneficioso hacer que los individuos del otro sexo inviertan más, ya que el esfuerzo que hace un individuo, es esfuerzo que el otro se evita (Aloise King et al. 2013). Bajo estricta monogamia los intereses de las hembras y machos generalmente convergen, ya que es importante el bienestar de ambos para el éxito reproductivo futuro (Holland & Rice 1999); sin embargo, en sistemas polígamos, ya que los individuos cambian de pareja reproductiva en cada evento, los intereses de hembras y machos giran en torno a la adecuación de cada uno y la intensidad del conflicto aumenta considerablemente (Aloise King et al. 2013).

La evolución de la viviparidad, y más particularmente de la matrotrofía, en sistemas poligámicos (y más estrictamente poliandricos), creó el escenario y las condiciones ideales para que el conflicto relacionado a la asignación materna de recursos durante el desarrollo embrionario tuviera lugar. En animales ovíparos, ya que la madre pone una cantidad fija de recursos en el huevo, la inversión materna sólo puede ser influenciada antes del apareamiento (e.g. con señales visuales o químicas de calidad del macho; Rich & Hurst 1998; Velando et al. 2006); sin embargo, una vez que el cascarón es puesto en el huevo, ni el macho ni los zigotos pueden manipular la asignación materna de recursos. En condiciones de viviparidad lecitrotrófica, al igual que en la oviparidad, la cantidad de

recursos maternos para los críos en desarrollo es determinada y fija (Blackburn 2000). Sin embargo, cuando la asignación materna de recursos es modificable durante el desarrollo embrionario, y la cantidad de recursos que la madre le transmite a sus críos depende de las necesidades de los mismos (que son transmitidas mediante una comunicación fisiológica madre-crío), si las señales que forman parte de la comunicación madre-crío son deshonestas, la madre puede transmitir más nutrientes de los que los críos realmente necesitan.

La cantidad de recursos maternos que es óptima para la madre y para sus críos normalmente difieren. Por ello, si surgen adaptaciones en los críos que favorezcan que su madre invierta una mayor cantidad de recursos de lo que sería óptimo para ella, se espera que este conflicto también lleve a contra- adaptaciones en la madre que le permitan controlar el suministro de nutrientes (Trivers 1974). Cuando el conflicto se da entre hembras y machos, o entre allelos de origen paterno y materno en el crío, se espera que las adaptaciones de origen paterno evolucionen para extraer una mayor cantidad de recursos, y que las adaptaciones maternas favorezcan una repartición equitativa de recursos a lo largo de los diferentes eventos reproductivos de las hembras (Haig & Westoby 1989).

A veces es difícil discernir qué tipo de conflicto está moldeando la evolución de las diferentes adaptaciones y contra- adaptaciones de los organismos, ya que a veces los intereses de más de dos individuos pueden diferir con respecto a una misma característica, y por lo tanto varios tipos de conflicto podrían estar jugando un papel aditivo en tal proceso (como por ejemplo el conflicto madre-crío y el conflicto sexual en torno a la asignación materna de recursos), o un tipo de conflicto podría estar englobado en el otro, como podría ser el caso del conflicto sexual y el conflicto entre alelos parentales.

Crespi & Semeniuk (2004) puntualizaron que cuando hay interacciones madre-crío existen 4 tipos de genes: genes expresados en la madre, genes expresados en el crío sin importar su origen parental, genes derivados maternamente expresados en el crío, y genes

derivados paternamente expresados en el crío. Los dos primeros tipos de genes muy probablemente son los que regulan el conflicto madre-crío; sin embargo, cuando los genes se expresan dependiendo del origen del alelo parental, es decir están impresos, esto más bien parece ser un rasgo distintivo de la selección favoreciendo un conflicto: 1) entre los intereses de los alelos parentales en el crío, donde en los alelos paternos se va a favorecer que los críos extraigan una mayor cantidad de recursos de la madre, y en los maternos una distribución equitativa de recursos entre las diferentes camadas (ver Haig 2000), o 2) de un conflicto entre machos y hembras con respecto a cuánto deben invertir las hembras en la camada en común durante su desarrollo embrionario. Estos dos últimos tipos de conflicto coinciden en que ambos pueden darse a nivel de expresión genética, y por lo tanto puede llevar a la impronta genética de genes que regulan el suministro de nutrientes maternos.

La asignación materna puede ser manipulada mediante la sobre expresión de genes responsables de la comunicación madre-crío para la transferencia de nutrientes maternos, como es el caso del factor de crecimiento tipo insulina 2 (*igf2* por sus siglas en inglés), que se encarga de regular la demanda de nutrientes por parte del crío y el suministro de nutrientes maternos por parte de la placenta (DeChiara et al. 1991). Este gen está impreso en mamíferos vivíparos, es decir, sólo el alelo paterno se expresa en la placenta y en los embriones, mientras que el alelo materno permanece silenciado (DeChiara et al. 1991; O'Neill et al. 2000). En este grupo, la impronta genética de *igf2* probablemente refleja el resultado de un conflicto evolutivo entre los alelos parentales en el crío, o entre los sexos, que inició probablemente con la sobre expresión del alelo paterno, que le permitió a sus críos recibir una mayor cantidad de nutrientes maternos y así nacer con una ventaja adaptativa que incrementara su adecuación, y que terminó con el eventual silenciamiento del alelo materno, lo cual favoreció una distribución óptima de recursos entre las diferentes camadas a lo largo de su vida reproductiva (Haig 2004; Wilkins & Haig 2001).

La evolución de la impronta genética de *igf2*, un gen que promueve el crecimiento, e *igf2r*, un gen que lo suprime, parece ser exclusiva de los mamíferos vivíparos (O'Neill et al. 2000; DeChiara et al. 1991; Nolan et al. 2001; J. Keith Killian et al. 2001; Killian et al. 2000) sin embargo, la viviparidad también ha evolucionado en peces, reptiles, anfibios (Blackburn 2015), donde las adaptaciones maternas y embrionarias han hecho posible que las hembras tengan un mayor control del suministro de nutrientes maternos, pero también que los embriones (o sus alelos derivados paternamente) puedan influir en la asignación materna de recursos (ver capítulo 1). Por ello, es posible que también en estos otros taxa haya surgido un conflicto en torno a la asignación materna de recursos durante el desarrollo embrionario.

Si el conflicto que evolucionó en mamíferos vivíparos también evolucionó en otros taxa, estos deben de compartir los atributos de poliginia, viviparidad materna. Adicionalmente, ya que la manipulación es fisiológica, entonces también deben de existir genes expresados en los críos, cuyos productos de la traducción sean capaces de influir en la distribución materna de recursos (Wilkins & Haig 2003).

Los goodeinos son un grupo de peces vivíparos matrotróficos, cuyas características de placentotrofía han permitido que a través de la placenta trofotenial los críos incrementen su peso seco considerablemente durante el desarrollo embrionario (Lombardi & Wourms 1988). Esta placenta tiene un componente embrionario y uno materno (Lombardi & Wourms 1985a; Lombardi & Wourms 1985b), lo que incrementa la posibilidad de una manipulación de recursos maternos mediante hormonas y/o tejidos embrionarios. Para determinar si el conflicto que hasta ahora se cree exclusivo de mamíferos, también ha evolucionado en peces vivíparos, primero debíamos determinar si la asignación materna de recursos puede ser modificada por los machos y/o los críos en desarrollo.

¿Los machos o los críos son capaces de influir en la asignación materna de recursos?

Una manera de determinar si existe algún tipo de conflicto entre machos y hembras es cruzar individuos de diferentes poblaciones que han evolucionado en aislamiento, y que por lo tanto se encuentran en un punto diferente de esa coevolución antagonista (Rowe et al. 2003; ver Vrana et al. 1998; Rogers & Dawson 1970). Nosotros utilizamos peces de las dos poblaciones genéticamente y geográficamente más distantes del pez Amarillo (*Girardinichthys multiradiatus:* Macías Garcia et al. 2012) que son Zempoala –Z- y San Marías el Grande –M-. Nuestros resultados mostraron un incremento asimétrico en la inversión de las hembras de ambas poblaciones cuando se aparearon con un macho de la otra población. Cuando cruzamos a una hembra de Z con un macho de M, los críos de las camadas de ese cruce fueron más largos, más anchos (es decir más grandes), y más pesados que los críos de su cruce control (Z-Z; población de la hembra X población del macho). Sin embargo, cuando los críos provinieron del cruce recíproco (M-Z), fueron estadísticamente del mismo tamaño y del mismo peso que los de su cruce control (M-M). Además en ambos cruces experimentales (Z-M y M-Z) el tamaño de la camada fue estadísticamente igual al de las camadas de sus cruces control (Z-Z y M-M respectivamente), lo cual respalda el hecho de que las hembras de Z sí están invirtiendo más recursos en los críos cuando se aparean con un macho de M.

Es poco probable que estos resultados fenotípicos hayan sido consecuencia de que las hembras de Z hayan encontrado más atractivos a los machos de M, ya que el patrón de cortejo, que es esencial para que las hembras acepten copular con el macho, difiere entre poblaciones, y por lo tanto actúa como barrera precopulatoria (González Zuarth & Macías Garcia 2006). Se ha demostrado que cuando los críos de diferentes poblaciones crecen juntos dicha barrera se puede superar, ya que los machos ajustan su cortejo y logran que las hembras aceptan aparearse con ellos; pero esto no incrementa la aceptación de las hembras con respecto a la aceptación que muestran a los machos de su población (De Gasperin & Macías Garcia 2014).

Estos resultados tampoco pueden ser explicados por heterosis o por depresión exogámica. La heterosis (o vigor híbrido; Shull 1908) puede producir efectos en la F1 que incrementen su adecuación (Edmands 1999). La depresión exogámica, por el contrario, puede afectar la adecuación de individuos híbridos como consecuencia de la disrupción de la coadaptacion de sus genotipos (Templeton et al. 1986). Nosotros observamos efectos en el cruce intra-poblacional Z-M que podrían encajar con los esperados por heterosis. Con respecto a la depresión exogámica, no vimos ningún efecto en la supervivencia de los críos o en la disminución en su tamaño, y aunque no realizamos más cruzas en generaciones subsecuentes, ni tampoco retro-cruzas para confirmar si la depresión exogámica estaba teniendo algún tipo de un efecto en la adecuación de los críos, los efectos de la heterosis y de la depresión exogámica suelen ser simétricos sin importar la dirección de la cruza (ver Edmands 1999), lo cual no sucedió en nuestro experimento.

Cuando se da una disrupción de los genotipos, y estos involucran genes impresos, por otro lado, la obtención de fenotipos aberrantes, sin importar la direccionalidad de la cruza, es posible. Esta disrupción puede llevar a la pérdida de la impronta genética, o a una expresión genética aberrante de genes relacionados al desarrollo y al crecimiento (ver Wolf et al. 2014), que son justamente los que pueden provocan incrementos en tamaño y/o peso de los críos.

Nuestros resultados son también consistentes con teoría del conflicto madre-crío (Crespi & Semeniuk 2004), y más fuertemente con el conflicto entre sexos (Parker 1979; Parker 2006), que ha favorecido adaptaciones en los críos para que estos extraigan una mayor cantidad de recursos de la madre y así consecuentemente la adecuación del macho aumente. Esto probablemente ha impulsado en las madres la evolución de contra adaptaciones que les han permitido mitigar los costos que les fueron impuestos por una inversión subóptima de recursos.

La eficacia de estas adaptaciones y contra adaptaciones parece ser propia de cada población. Nuestros resultados sugieren que los críos son mejores extrayendo recursos de su madre cuando su padre proviene de M, lo cual podría ser consecuencia de que alelos paternos de genes que pueden influir en la asignación materna de recursos han evolucionado para favorecer una extracción de recursos más efectiva en M que en Z. Alternativamente, es posible que las contra- adaptaciones de las hembras de M para evitar una manipulación de recursos maternos sean más eficaces que las de las hembras de Z, o quizás una combinación de ambos.

Nuestros datos nos permiten descartar la heterosis y la depresión exogámica como posibles explicaciones para nuestros resultados; sin embargo, no nos es posible determinar si la manipulación de los machos o la disrupción de la coadaptación de los genes impresos son los responsables de los resultados fenotípicos asimétricos que observamos en los críos de los cruces Z-M y M-Z. Una manera de tener un acercamiento al fenómeno que originó nuestros resultados es determinar si al igual que en mamíferos, la expresión del gen que codifica para el factor de crecimiento tipo insulina 2 (*igf2* por sus siglas en inglés) es dependiente de su origen parental (DeChiara et al. 1991). Las señales que gobiernan la impronta genética parecen estar evolucionando muy rápidamente dentro de cada especie (o población aislada) y pueden producir resultados similares a los nuestros en cruces de individuos que difieren en las características propias de la impronta genética (Vrana et al. 1998). Además, se ha demostrado que las bases de la impronta genética de *igf2* están en gametos de peces ovíparos (Xie et al. 2009), lo que significa que este gen podría estar impreso también en peces vivíparos matrotróficos como consecuencia del conflicto entre los alelos parentales (Haig & Westoby 1989) o entre los sexos (Chapman 2006; ver Houston et al. 2005) con respecto a la asignación materna de recursos durante el desarrollo embrionario.

¿Qué papel tiene el gen *igf2* en peces vivíparos, cuáles son sus características y qué fuerzas evolutivas están moldeando su evolución?

El factor de crecimiento tipo insulina 2 es una proteína semejante a la proinsulina que juega un papel muy importante durante el desarrollo embrionario (Schmid 1995). En mamíferos, una de sus principales funciones es controlar la demanda de nutrientes por parte del feto y el suministro de nutrientes por parte de la placenta (DeChiara et al. 1991); sin embargo, en peces este gen también cumple una gran variedad de funciones relacionnadas al desarrollo (Wood et al. 2005). Además, *igf2* es expresado en todos los tejidos de embriones de peces ovíparos (Yuan et al. 2011), y en peces placentados su secuencia ha evolucionado bajo selección direccional como resultado del conflicto madre crío (O'Neill et al. 2007). En los goodeinos, al igual que en la mayoría de los teleósteos (Wood et al. 2005), este gen está conformado por 4 exones y 3 intrones, que juntos tienen un marco de lectura abierto (Open Reading Frame -ORF- por sus siglas en inglés) de cerca de 5 kb, y una secuencia codificante (CDS) de alrededor de 645-650 pb.

Nosotros detectamos una gran cantidad de SNP dentro de la secuencia de *G. multiradiatus*. Sorprendentemente, de los 5 SNPs que localizamos dentro de la CDS, 4 de ellos se encontraron en el exón 2, lo cual sugiere que el exón 2 podría estar jugando algún papel en la regulación de la expresión génica, en la conformación del mRNA, o en la eficiencia de traducción (Komar 2003).

El factor de crecimiento tipo insulina 2, al ser una proteína con una función tan importante y conservada dentro de los vertebrados, mantiene un porcentaje de identidad alto, incluso entre diferentes especies (ver Wood et al. 2005). Dentro de los teleósteos, el porcentaje de identidad entre secuencias mRNA y proteínas fue también muy alto (superior al 70%), y dentro de los goodeinos nunca fue menor al 90% (ver capítulo 5 para más detalles).

Como esperábamos, el porcentaje de identidad de las secuencias de *igf2* de diferentes especies de goodeinos está relacionado con qué tan emparentadas están filogenéticamente; sin embargo, nuestros resultados sugieren que la evolución de la secuencia de este gen se relaciona más con la intensidad de la selección sexual dentro de cada especie más que con la distancias filogenéticas entre ellas.

El conflicto sexual relacionado a la reproducción puede darse en torno a atributos que evolucionaron por selección sexual y que imponen un costo a los portadores cuando son indicadores de calidad del individuo (Zahavi 1975), o a diferencias en los intereses entre machos y hembras con respecto al apareamiento y a la fertilización (Parker 2006). En estas especies, el cortejo es una actividad energéticamente demandante (De Gasperin & Macías Garcia 2014), y el dicromatismo, cuando se da por la acumulación de carotenoides, también es fisiológicamente costoso de mantener (Grether et al. 2001). De las secuencias de *IGF2* de goodeinos que analizamos, las que mostraron un mayor porcentaje de identidad, no fueron las especies más cercanamente emparentadas sino las que tienen valores de dicromatismo y complejidad en su cortejo similares. *Girardinichthys viviparus*, a pesar de ser especie hermana de *G. multiradiatus*, tiene una secuencia de aminoácidos más similar a la de *Ilyodon furcidens*, que es una especie filogenéticamente lejana (Webb et al. 2004), pero que tiene un valor bajo de dicromatismo y un patrón de cortejo igual de sencillo al de *G. viviparus.* Las secuencias de aminoácidos de *G. multiradiatus* y *Allotoca diazi*, que son especies con valores semejantes de dicromatismo y complejidad de cortejo, también fueron muy similares. Su secuencia comparte el 97.14% de identidad, uno de los valores más altos entre todas las secuencias analizadas.

La alta similitud entre las secuencias de *igf2* entre especies de peces lejanamente emparentados brinda soporte a la idea de que este gen es sumamente importante para el desarrollo embrionario no solo en mamíferos, sino también en teleósteos (Wood et al. 2005; Duan 1997); sin embargo, aunque la secuencia del gen está bajo una presión de selección

debida al conflicto madre-crío en teleósteos placentados (O'Neill et al. 2007), aparentemente fuerzas evolutivas adicionales, como por ejemplo la que surge como producto del conflicto que se deriva entre los sexos en torno a la selección sexual, están imponiendo también una presión de selección en la evolución de la secuencia de este gen en los goodeinos.

Nosotros desconocemos los efectos que todas las variaciones en las secuencias de gDNA, mRNA y aminoácidos tienen en la producción y conformación de la proteína; sin embargo, nuestros resultados sugieren que la evolución *igf2* parece estar fuertemente dirigida por diversos tipos de conflicto en los goodeinos, llevando a algún tipo de convergencia en la secuencia de aminoácidos de la proteína. Por ello, es importante realizar más investigación para ahondar en el papel que juega este gen en el desarrollo de estos peces, y también para tener un acercamiento del efecto que pueden tener en el *IGF2* maduro de las diferentes especies todas las variaciones y SNPs que detectamos.

¿*Igf2* tiene un patrón de expresión alélica dependiente del origen parental en peces vivíparos?

Los resultados fenotípicos que obtuvimos sugieren que la asignación materna de las hembras de Z puede ser manipulada cuando se aparean con los machos de M. Adicionalmente, el gen *igf2* parece ser un gen que está bajo diversas presiones de selección derivadas del conflicto dentro de la familia. Si la expresión genética de *igf2* está siendo moldeada por un conflicto entre los intereses de hembras y machos, o de los alelos derivados materna y paternamente en el crío, entonces esperaríamos que sólo el alelo paterno se expresara en los embriones de goodeinos.

Las secuencias exónicas de *igf2* de Z y M, las poblaciones genéticamente más distantes de *G. multiradiatus*, tienen una similitud notable (para más detalles ver capítulo 5). Debido a esto, la única estrategia que nos permitió determinar si *igf2* se expresa monoalélica o bialélicamente en embriones de *G. multiradiatus*, fue encontrar una pareja

reproductiva en la que los individuos difirieran en algún SNP que nos permitiera distinguir ambos alelos parentales en críos heterocigotos; sin embargo, el único SNP que se mostró constante en la población, tuvo una distribución totalmente sesgada hacia una de las variantes alélicas (T), y sólo pudimos detectar la presencia de la otra variante alélica en individuos heterocigotos (T/C). Por ello, la pareja que utilizamos estaba conformada por una hembra heterocigota (T/C) y un macho homocigoto (T/T). Los embriones heterocigotos (que habían heredado la variante alélica T de su padre y la variante alélica C de su madre) aparentemente sólo mostraron expresión de *igf2* paterna, ya que de las 78 y 54 secuencias que obtuvimos todas correspondían al alelo paterno (T). Esto podría sugerir que, al igual que en mamíferos, *igf2* está impreso en embriones de goodeinos, donde solo la copia paterna se expresa y la materna permanece silenciada; sin embargo, dada la escasez tanto de parejas que nos permitieran rastrear los alelos parentales en los críos, como de críos heterocigotos, nos fue imposible realizar más experimentos que enrobustecieran nuestros resultados y que nos permitieran confirmar la expresión monoalélica de *igf2*.

Aunque no pudimos describir el patrón de expresión de *igf2* en los goodeinos, encontramos un patrón de metilación diferencial en una región de *igf2* que incluye al exón 2 y una sección de los intrones adyacentes. El silenciamiento de genes se da normalmente por la adición de grupos metilo al carbono 5 de las citosinas que están en dinucleótidos CpG (Hendrich & Tweedie 2003). Dentro de las secuencias que analizamos provenientes de un embrión heterocigoto (T/C) (del cual conocíamos el origen de cada alelo parental), y de una hembra adulta (T/T), de la que desconocíamos qué alelo era el paterno y cual el materno, observamos claramente dos patrones de metilación diferencial propios de cada alelo, donde uno estaba hipometilado y el otro hipermetilado. En el embrión, el alelo materno fue el que mostró una gran cantidad de citosinas metiladas (en contexto CpG y fuera de contecxto CpG), y por lo tanto esto brinda soporte a la posibilidad de que el alelo materno de *igf2* esté siendo silenciado epigenéticamente en embriones de *G. multiradiatus*. Además,

este patrón de metilación diferencial en el exón 2 coincide con la gran cantidad de polimorfismos que encontramos en esa región (ver arriba), lo que brinda más soporte a que esa región está jugando algún papel en la regulación de la expresión de *igf2*; sin embargo, es necesario realizar más estudios que nos permitan ahondar en el patrón de expresión y el mecanismo de expresión de *igf2.*

La evolución de la impronta genética de *igf2* se ha buscado en otro grupo de peces vivíparos placentados muy cercanamente emparentados a los goodeinos, donde los autores encontraron que *igf2* se expresa bialélicamente (Lawton et al. 2005). Nosotros tenemos evidencia que sugiere que *igf2* se expresa monoalélicamente en goodeinos. A pesar de que los poeciliidos y los goodeinos son ambos Cyprinodontiformes, los goodeinos tienen ciertos atributos o características que pueden ser determinantes en la intensidad del conflicto que pudo haber favorecido la evolución de la impronta de *igf2*. En los goodeinos, para que las hembras acepten el apareamiento los machos deben de realizar despliegues de cortejo en forma de danzas que pueden llegar a ser muy elaboradas y costosas energéticamente (De Gasperin & Macías Garcia 2014). Este atributo, que ha evolucionado sólo en la mitad de las especies de poeciliidos (Pollux et al. 2014), aparentemente está influyendo en la evolución de *igf2* dentro de la familia (ver arriba), posiblemente a causa de un conflicto entre los intereses de hembras y machos con respecto a la reproducción. Adicionalmente, el tiempo de gestación es dos veces más largo en los goodeinos que en los poecíliidos (8 semanas vs 4 semanas respectivamente), lo que permite, junto con la alta eficiencia de la placenta trofotenial en la transferencia de nutrientes maternos, que los embriones de goodeinos alcancen un incremento en peso seco de hasta 15 000% (Ameca splendens; Lombardi & Wourms 1988), comparado con el de los poeciliidos de 4000% (*Heterandria formosa*; Wourms 1981).

Por otro lado, aunque no se han dedicado más esfuerzos a indagar si la impronta genética de genes como *igf2* ha evolucionado en otros grupos de peces vivíparos, las bases

de la impronta genética de *igf2* está documentada en gametos de peces ovíparos (Xie et al. 2009), y además tanto *igf2* como *igf2r* se expresa en peces. Además, el *igf2* de peces exhibe una estructura y una afinidad por *IGF2* similares a las de su gen ortólogo en mamíferos (Méndez et al. 2001), y desempeña también un papel en la degradación de *igf2* (Nolan et al. 2006). Todo lo anterior, junto con las características de matrotrofía y placentotrofía que les permite a los embriones recibir una gran cantidad de recursos maternos a través de la placenta trofotenial (Lombardi & Wourms 1985a; Lombardi & Wourms 1985b; Schindler 2003), y con el sistema de apareamiento poligámico que caracteriza a la mayoría de los peces, hacen que los goodeinos cumplan con todas las condiciones propuestas por Wilkins y Haig (2003) para que la impronta genética de *igf2* evolucione.

La teoría del parentesco, que hasta ahora es la teoría más aceptada para la evolución de la impronta genética, fue desarrollada por Haig y colaboradores (Moore & Haig 1991; Haig 2000; Haig & Graham 1991; Haig & Westoby 1989) y propone que la impronta genética de genes como *igf2* e *igf2*r evolucionó como resultado un conflicto entre los alelos parentales dentro del crío con respecto a la asignación materna de recursos durante el desarrollo embrionario (Haig 2000a; Moore & Haig 1991); sin embargo, es tal la diversidad de genes impresos dentro del genoma (151 en ratón hasta ahora ; Williamson et al. 2013), que esto ha llevado a Wolf y Hager (2006) a proponer que la impronta genética parece haber evolucionado debido a diferentes presiones de selección en diferentes loci. Si esto es cierto, también es posible que más de una fuerza selectiva, o de un tipo de conflicto, pueda haber moldeando la evolución de un gen como *igf2*. Aunque el la teoría de Haig hace énfasis en que el conflicto es únicamente entre alelos parentales en el crío, nosotros consideramos que al menos dentro de nuestro sistema de estudio, y probablemente en muchos otros organismos, el conflicto sexual, que desde nuestra perspectiva engloba al de los alelos parentales, puede estar ejerciendo una presión de selección que esté dirigiendo

la evolución de *igf2*, y quizás de otros genes similares y/o con efectos antagónicos, en un tipo de coevolución antagonista como la de la "*Interlocus Contesto Evolution*"(Rice & Holland 1997).

En los goodeinos el conflicto sexual relacionado al apareamiento ha sido demostrado (Macías Garcia & Ramirez 2005; Garcia et al. 2012). Además, dados los costos tan altos que pagan hembras y machos en cada época y evento reproductivo (e.g. costos relacionados a la viviparidad y los derivados de la selección sexual), es posible que todo este conflicto de intereses esté afectando la evolución de varias características dentro de la familia, y por lo tanto podría estar jugando un papel importante en la evolución (y posible impronta genética) de *igf2* dentro de los goodeidos. Nuestros resultados de expresión alélica no son concluyentes, pero eso no descarta la posibilidad de que la impronta genética también haya evolucionado en los goodeinos.

Conclusiones

La diferencia en la inversión de las hembras de Z y M cuando se aparearon con un macho de la otra población, aunque es asimétrica, puede significar que i) los machos, los críos, o ambos, son capaces de influir en la distribución materna de las hembras de *G. multiradiatus*, o ii) que el aumento en talla y peso de los críos del cruce Z-M son producto de una alteración en la coadaptación entre los genotipos (incluyendo genes impresos) de madres y críos.

Nuestros datos no nos permitieron discernir entre ambas posibilidades; sin embargo, *G. multiradiatus* es un pez vivíparo matrotrófico, con un sistema de apareamiento poligámico, y cuyos embriones expresan *igf2* durante la gestación. Además, la evolución en la secuencia de mRNA de *igf2* dentro de los goodeinos parece estar siendo influida por un conflicto entre hembras y machos producto de la selección sexual. Adicionalmente, aunque no pudimos determinar si *igf2* está impreso en esta especie, los resultados de la secuenciación con bisulfito sugieren que *igf2* es regulado epigenéticamente. Por todo lo anterior, consideramos que nuestros resultados soportan más la hipótesis del conflicto,

donde los padres, o los padres y los críos, son capaces de influir en la asignación materna de recursos durante el desarrollo embrionario; sin embargo, este conflicto que aparentemente causó que surgieran adaptaciones en los machos, aunque son expresadas en los críos, parece haber llevado a la evolución de contra adaptaciones en las hembras (también expresadas en los críos) para resistir dicha manipulación. El gen *igf2*, y quizás otros genes con funciones similares y/o antagónicas (como *igf2r*), podrían estar desempeñando algún papel un papel en dicha coevolución antagonista, y por ello es necesario estudiar ese fenómeno con más detalle en otros grupos vivíparos placentados.

Bibliografía

Aloise King, E.D., Banks, P.B. & Brooks, R.C., 2013. Sexual conflict in mammals: Consequences for mating systems and life history. *Mammal Review*, 43(1), pp.47–58.

Blackburn, D.G., 2000. Classification of the Reproductive Patterns of Amniotes. *Herpetological Monographs*, 14, pp.371–377.

Blackburn, D.G., 2015. Evolution of vertebrate viviparity and specializations for fetal nutrition: A quantitative and qualitative analysis. *Journal of Morphology*, 276(8), pp.961–990.

Chapman, T., 2006. Evolutionary Conflicts of Interest between Males and Females. *Current Biology*, 16(17), pp.744–754.

Crespi, B. & Semeniuk, C., 2004. Parent-offspring conflict in the evolution of vertebrate reproductive mode. *Am Nat*, 163(5), pp.635–53.

DeChiara, T.M., Robertson, E.J. & Efstratiadis, A., 1991. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell*, 64(4), pp.849–859.

Duan, C., 1997. The insulin-like growth factor system and its biological actions in fish. *Amer. Zool.*, 37, pp.491–503.

Edmands, S., 1999. Heterosis and Outbreeding Depression in Interpopulation Crosses Spanning a Wide Range of Divergence. *Evolution*, 53(6), pp.1757–1768.

Garcia, C.M. et al., 2012. Foraging costs drive female resistance to a sensory trap Subject collections Foraging costs drive female resistance to a sensory trap. , (February).

De Gasperin, O. & Macías Garcia, C., 2014. Congenital predispositions and early social experience determine the courtship patterns of males of the Amarillo fish. *Behavioral Ecology and Sociobiology*, 68(4), pp.639–648.

González Zuarth, C. & Macías Garcia, C., 2006. Phenotypic differentiation and pre-mating isolation between allopatric populations of Girardinichthys multiradiatus. *Proceedings. Biological sciences / The Royal Society*, 273(1584), pp.301–7.

Grether, G.F., Hudon, J. & Endler, J. a, 2001. Carotenoid scarcity, synthetic pteridine pigments and the evolution of sexual coloration in guppies ([i]Poecilia reticulata[/i]). *Proceedings. Biological sciences / The Royal Society*, 268(1473), pp.1245–1253.

Haig, D., 2004. Genomic imprinting and kinship: how good is the evidence? *Annual review of genetics*, 38, pp.553–585.

Haig, D., 2000. The Kinship Theory of Genomic Imprinting. *Annual Review of Ecology and Systematics*, (34), pp.9–32.

Haig, D. & Graham, C., 1991. Genomic Imprinting and the Strange Case of the Insulin-like Growth Factor II Receptor. *Cell*, 64, pp.1045–1046.

Haig, D. & Westoby, M., 1989. Parent-Specific Gene Expression and the Triploid Endosperm. *The American Naturalist*, 134(1), p.147.

Hendrich, B. & Tweedie, S., 2003. The methyl-CpG binding domain and the evolving role of DNA methylation in animals. *Trends in Genetics*, 19(5), pp.269–277.

Holland, B. & Rice, W.R., 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proceedings of the National Academy of Sciences of the United States of America*, 96(9), pp.5083–5088.

Houston, A.I., Székely, T. & McNamara, J.M., 2005. Conflict between parents over care. *Trends in Ecology and Evolution*, 20(1), pp.33–38.

Killian, J.K. et al., 2000. M6P/IGF2R imprinting evolution in mammals. *Molecular cell*, 5(4), pp.707–716.

Killian, J.K. et al., 2001. Monotreme IGF2 expression and ancestral origin of genomic imprinting. *Journal of Experimental Zoology*, 291(2), pp.205–212.

Komar, A.A., 2003. *Single Nucleotide Polymorphisms* Second Edi. A. A. Komar, ed., Cleveland: Humana Press.

Lawton, B.R. et al., 2005. Allelic expression of IGF2 in live-bearing, matrotrophic fishes. *Development Genes and Evolution*, 215, pp.207–212.

Lombardi, J. & Wourms, J.P., 1988. Embryonic growth and trophotaenial development in goodeid fishes (Teleostei: Atheriniformes). *Journal of Morphology*, 197(2), pp.193–208.

Lombardi, J. & Wourms, J.P., 1985a. The trophotaenial placenta of a viviparous goodeid fish. I: Ultrastructure of the Internal Ovarian Epithelium, the Maternal Component. *Journal of molecular biology*, 184, pp.277–292.

Lombardi, J. & Wourms, J.P., 1985b. The trophotaenial placenta of viviparous goodeid fish. II. Ultrastructure of the trophotaenia, the embryonic component. *Journal of Morphology*, 184(3), pp.293–309.

Macías Garcia, C. et al., 2012. Variation in sexual dimorphism and assortative mating do not predict genetic divergence in the sexually dimorphic Goodeid fish Girardinichthys multiradiatus. *Current Zoology*, 58(3), pp.440–452.

Macías Garcia, C. & Ramirez, E., 2005. Evidence that sensory traps can evolve into honest signals. *Nature*, 434(7032), pp.501–505.

Méndez, E. et al., 2001. Identification of a type II insulin-like growth factor receptor in fish embryos. *Endocrinology*, 142(3), pp.1090–1097.

Moore, T. & Haig, D., 1991. Genomic imprinting in mammalian development: a parental tug-of-war. *Trends in Genetics*, 7(2), pp.45–49.

Nolan, C.M. et al., 2001. Imprint status of M6P/IGF2R and IGF2 in chickens. *Development Genes and Evolution*, 211(4), pp.179–183.

Nolan, C.M. et al., 2006. Mannose 6-phosphate receptors in an ancient vertebrate, zebrafish. *Development Genes and Evolution*, 216, pp.144–151.

O'Neill, M.J. et al., 2000. Allelic expression of IGF2 in marsupials and birds. *Development genes and evolution*, 210, pp.18–20.

O'Neill, M.J. et al., 2007. Ancient and continuing Darwinian selection on insulin-like growth factor II in placental fishes. *Proceedings of the National Academy of Sciences of the United States of America*, 104(30), pp.12404–12409.

Parker, G.A., 2006. Sexual conflict over mating and fertilization: an overview. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 361(1466), pp.235–259.

Parker, G.A., 1979. Sexual selection and sexual conflict. In M. Blum & N. Blum, eds. *Sexual Selection and Reproductive Competition in Insects*. New York: New York Academy Press, pp. 123–166.

Pollux, B.J.A. et al., 2014. The evolution of the placenta drives a shift in sexual selection in livebearing fish. *Nature*, 513, pp.233–236.

Rice, W.R. & Holland, B., 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behav Ecol and Sociobiol*, 41, pp.1–10.

Rich, T. & Hurst, J., 1998. Scent marks as reliable signals of the competitive ability of mates. *Animal behaviour*, 56(Drickamer 1992), pp.727–735.

Rogers, J.F. & Dawson, W.D., 1970. Foetal and placental size in a Peromyscus species cross. *J Reprod Fertil*, 21(2), pp.255–262.

Rowe, L., Cameron, E. & Day, T., 2003. Detecting sexually antagonistic coevolution with population crosses. *Proc. R. Soc. Lond. B*, 270, pp.2009–2016.

Schindler, J.F., 2003. Scavenger Receptors Facilitate Protein Transport in the Trophotaenial Placenta of the Goodeid Fish, Ameca splendens (Teleostei : Atheriniformes). *Journal of Experimental Zoology*, 212(May), pp.197–212.

Schmid, C., 1995. Insulin-like growth factors. *Cell Biology International*, 19(5), pp.445–457.

Shull, G.H., 1908. The composition of a field of Maize. *Journal of Heredity*, 4(1), pp.296–301.

Templeton, A.R. et al., 1986. Local Adaptation , Coadaptation , and Population Boundaries. *Zoo Biology*, 5, pp.115–125.

Trivers, R.L., 1974. Parent-Offspring Conflict. *American Zoologist*, 14(1), pp.249–264.

Velando, A., Beamonte-Barrientos, R. & Torres, R., 2006. Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. *Oecologia*, 149(3), pp.535–542.

Vrana, P.B. et al., 1998. Genomic imprinting is disrupted in interspecific Peromyscus hybrids. *Nature genetics*, 20(december), pp.362–365.

Webb, S.A. et al., 2004. Molecular phylogeny of the livebearing Goodeidae (Cyprinodontiformes). *Molecular Phylogenetics and Evolution*, 30(3), pp.527–544.

Wilkins, J.F. & Haig, D., 2001. Genomic imprinting of two antagonistic loci. *Proceedings. Biological sciences / The Royal Society*, 268(1479), pp.1861–7.

Wilkins, J.F. & Haig, D., 2003. What good is genomic imprinting: the function of parent-specific gene expression. *Nature Reviews Genetics*, 4(5), pp.359–368.

Williamson, C.M. et al., 2013. World Wide Web Site - Mouse Imprinting Data and References -. *MRC Harwell, Oxfordshire*.

Wolf, J.B. & Hager, R., 2006. A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLoS Biology*, 4(12), pp.2238–2243.

Wolf, J.B., Oakey, R.J. & Feil, R., 2014. Imprinted gene expression in hybrids: perturbed mechanisms and evolutionary implications. *Heredity*, 113(2), pp.167–75.

Wood, A.W., Duan, C. & Bern, H. a., 2005. Insulin-like growth factor signaling in fish. *International Review of Cytology*, 243, pp.215–285.

Wourms, J.P., 1981. Viviparity: The maternal-fetal relationship in fishes. *Integrative and Comparative Biology*, 21(2), pp.473–515.

Xie, B. et al., 2009. The evolutionary foundation of genomic imprinting in lower vertebrates. *Chinese Science Bulletin*, 54(8), pp.1354–1360.

Yuan, X.-N. et al., 2011. Functional conservation and divergence of duplicated insulin-like growth factor 2 genes in grass carp (Ctenopharyngodon idellus). *Gene*, 470(2011),

pp.46–52.

Zahavi, A., 1975. Mate selection-a selection for a handicap. *Journal of theoretical biology*, 53(1), pp.205–214.

Zeh, J.A. & Zeh, D.W., 2008. Viviparity-driven conflict. *Annals of the New York Academy of Sciences*, 1133(1), pp.126–148.

Appendix 1. *Igf2* ORF of *Girardinichthys multiradiatus* (Zempoala population)

ATGGAGACCCAGCAAAGATCCGGACACCACTCACTTTGCCACACCTGCCGGACAACGGAGAGCTGCAGT
ATGAAGGTAACTGGAGCACAAATAGTTTCAGATTCCCCGTTTGAGCCGTGAGAAGGCTCAGGTAGAGCCG
ACTGGATAATTGTTGCGCAAAGGGGTACAGATAGATGCTTGTTGGCATCAGCTTTCTGCATTTTGAACAAG
CGGAATTTCTTGATTTGCATGCAGCAGAGGAAGTTCAGAATGCACAAACTGCAAAGGGAGACGCTTTGTG
TATTTATACCACGACAAGAAAGTAAAGAAATGAAAAGGTTATTTTTAATCTCCCCTTGATCTAAATAACGCG
CCGTTCGCCGGACACGGGCGCAGGAACAGCTGGTCGCCTTGCCGGCAGACAGGGGGCGGTAGGTCTGA
GGGTCATTGAGCTCACTCCGGGGTGAGCAAGGGGAAATTAAATGCTGAGTCTGTCTTTTTGGGGTAGTTA
ACTATCGAGCAGCCGGCCTTGCAACTTTCTGGGGTGGAGGGGGGAGTTGTGTAAGAATGAAAGCTTAGA
GATACTGGCGACTTTTTTCCTTCGGAATAAGCACTTCTCCCAGGTGCCAAACACATGACAGCAGCTGAAAA
AAATCGGGTCCTTGGTGTGTGTTTGAATGTGTTTATTTGATTTGATTTGACTCGCAGCGCACACTTTTTTCC
CCGAGTTACCAGGTCAGTGCGTGAAACAGCGTTTAAGACTTTAATCTCTTACATCTCGTACAAAACAAAAC
ATCTAGCTATTATTTGAGTTCTTTACATATAATTTTTATTGTCATAATAATGGATCACAAGTCTAACATTTTTC
CGAATCTTATGCTTTAATTTGTGTGTTTCCCCCTGCAGGTCAAGAAGATGTGCGCGACCAGCCGTGCGCT
GCTCTTTGCGCTGACCCTCACGCTCTACGTTGTGGAAATGGCCTCGGCAGAGACGTGGTGTGGCGGAGA
GCTGGTGGATGTGCTGCAGTTTGTCTGCGAAGACAGAGGCTTCTATTTCAGTAGGTTTTTTTTTTTCAGA
GCTATGCAAGTTTCTCCAAAAACTAGCTGCGCAAATGTTGATTCGCCTACCTTTTTAATGTTATTCGCCCTT
TTCGCTCCCCTTCCTCTCTGTTGTTTCACATCTGTTTGGATAGCTGAGACTGTCTGTGGCTGCGTGGTATA
CGTGTTTGCTATTTTTCTTTCTCACGCACTGACGCTCATGTAACACACACACAATCGCACACACGCCCGTG
CGCGCAAACGGCGTCAGAGAGAGAGAGAGAGAGAGAGAGAGAGGGAGAGAGAGGGAGATTGCAGCCAGCT
GTGCCTCTCCAGGTCTGCTCTGCTGAGTCTGCTCACAGCCTGCACCTCTCATTGCATATGAAAGGGCCAG
GCGGGCGAGCTGCAAAGGAACGTGCACGACCCCGGCGTCACAATGGGCTGCTTGTTTATCAGTAAAGTA
ATAGCACCCCAAGAGGCATTACCAGTCACCTCAGCACCAGTATTCCCAGGCCAGGAGACCACACTGATGA
GCTGGGAGGAACGAAAGAAATAAAGAGAGAGAGAGAGAGAGAGATCGTTGTCAGGATGAGAGAGAAAAATA
AAATCAACATTTTATTATTTCTGATAGTTGCTTCCATAATATTGATGATGGGGTTGGCTGTATGGTTTTATGC
AGAGCATTGTCGTTAATCCTCAACTGGATAATCTTCTGTCTGCTTAGTCTGCGTTGCACGTGATATTCATAA
CTTTCCTGTTCTTCTGATTGCTCCCCGTTTTGCGTTTAAAAATACTGGCCACATTTAGGTATGGAGTGTTAA
AACTTACCACAAAATATTTTTTCATTTTTACTGGTGCTAAAATAATTGAAAATATTGATTATTGCAGTTATAAT
AATGTTATATGTTAGTGGTTGCCAAAGTTTTTGCTGGGATCCTACTCGGGGCATGAATCAGGAAGTGTAGG
GTCTTGAGATTCTCTTTGGAAACCAAAGTAAAACACAAACATGATCCTATAACTACCACATGATGTAAAGAG
CTGTTGTTATACATAGCTAAAAGCGATAAAGTGGTTGCTGATGTTATTTGTGTTATCATTATACTCCTATTTC
ATTGGCTTATTGTTAAAGCTTGCGTTCTAAATCTTACAGTTGGGATCACAGCTCTCTTCCACTAATTATTTG
TGGGTCAGAAGGTGGAAGGTTTAGGAACCAGTTATATGTAAAACATTTGTTTATTATTCTTTTGAGCGGTTA
ATAAATGGACTAAAGCAAAAATCTAGAATGAATTCAATGCTAGTATTCAATTGCTTTGGGACCTTCTTTGCT
GTTGTGCTCTATATAGAGTAACACAAATGTAACAAGCTGATAAAAGCTGGTTGATTAGTCAGGCTGTTCAG
GTAGCCAGCCTGCATTCTGCTGCTTAACAGACCAACTGCAGTTAGAGAATGGCATCCATAGTTTGTGCAA
CTGGAAATATTTCCAAACATCCAAATGTGACATCAGTGTAGCAGCCTTCTTTCAGCTATCTGACTGTCCTTG
TGCAGCTTTAAACCATAATGATTGTCTGACTAAAAATTTCAGTGGTTTTGAAACCGGAACTGTTCAAAACCA
CAGTATATGTTGATACTGGTAACCAGCCCACCCATGCCTAGCTGTGCTCCCGCTTCTCAATTGCCTTTTGA
CAAAGAGCAGAATCTTTTGTTTATCAAAGAAAATTTAGTCAATCAGTCCTCCCTCTCCCTGGATGACCCAGT
CTTCCTGTAATCAAATCTAGCCAAGCCTCATGTGAGACTTTTCTAACTTAGTGTGGGCTGCCAGGCTTCAG
ACAAGGCCTTTATTGATCATGTGTAATGTTACCTTGTGGAAAACCCCTCACCTTTGCCTGCTATGTGTTACT
TCTTCTCTTGTTTTGGTTTTACTGTTCTTACTTTCTGTTTCTACCCATTCTTTGCCAATTTGACAAAGCAAAT
GAGTTTTAGATTTAAATATTTAATTATTTTGAAGAATTTCTGAATGCATTCTCTGGTTTCTGGTCACTTTGGA
CATGGTGTTTACTTTTAACACACTCATTTCGGGATGCCCAGATAGGCCTAATCAGATTATACCTTACAACCA
AAAATATTTGTTGCTGTTCGTCGCAGATTTCCTTTTATTTGTCAATGTAATCCTGCAAAAACAACCGTAATTC
GTTACTGTGGACAATGTGTGTTTTTTTGAAGGCCTCAACCTTCTATAACCTTTCTTACCGCTGTGTTTCTCC
TCAGGTAGGCCAACCAGCAGGGGTAACAACCGGCGGATCCAGACCCGTGGGATTGTAGAAGAGTGTTGT
TTCCGTAGCTGTGACCTCAACCTGCTGGAGCAATACTGTGCCAAACCCGCCAAGTCCGAGAGGGACGTTT
CGTCCTCCTCTATACAGGGCACGCCTGTTATGCCCACAACAAAGCAGGTGGGTCCAACCGGCAACCACA
ACACAAACTATAGTCCAGGTCTCTATCTGCCCTCTTATCTCACCTGAAACACAGAGTGCAGACTGAAATCC
ACACAAAAACAGGTTGATCTTGTTAAGTTCTCAGTGCTAAGCAGTTGTAAAATGCTGACCATGGTGGAAAA
CATTAGTGCAATCACAGTACATTATATTGTAGAAATAGCAGCGTCAATAATCTCATGGTGAAGTAGCTGAG
TGGTGAACCGGAAAGAAACAGCATGTTTTGATTGTCTGATAATCAGTCAAAGAACAGCAAGCTGTCTCAAA
ATATTCACATTCGAAAACACCACGTGTTTTTAGAAGAACTTTATTGGGAGGTGCCAGAAATGTGAATTAGCT
GAGACATCGTCAACACAATCTCTACATTTTCTGCTAATAATATGCTAAAAAGAAGTATAAATGCTGCAGACT
TAGAAAGCGATCTCTCACTATAACAGTAATTAAAAACAGGCTGCTGGGGAATTAGTTTGGATATGTCTCCA

GTTTGTCCCTGTGGGGAAAGTTGATGGTTGCAGGCTGCACTGAGGGCCAGCTGTTCTTGGGGCTATTGA
GCAGTTCTAGAGTGAATTAAGGGGGGTAAACACTCTTCAAGTGGCAGGACCAAAACAAGCTATTCACATG
GAGCACTGGCTACTCACGCATAAATCCTACCTAGCTCAGCTGCCAGCATTCCCACTTGTCCCAAAACAAC
ACCTACAACTGTTTGTCAGCATTAGCATAGATATTCTAAAATGAGTTTCCTTTGGTTGAGTGACAGCGTGCA
GTTAATATGTATATGAGGGGAGAATGGCCACCCTTCCTTTGTAATGGTATGCTAAAGAGGCCAAGCACTTA
TTGACAGCTTCTCTCACTTGAGGAAACCAAAACAAGGGCGGGCTGGGGTCGAGATGAGGTTGTTTTGATG
TTCACTGCTATAAGAATGAGATTTTATTTCTATTTTAGCAGCCCCAACAATGACCAAATGTTTTTGCGGAAT
AAGCAACACCTTACTCCATTGTAACGAGTTCTTGAACAGTAATCTTATCTCCGCTATTGAACTGACTTCCTC
TTCTCTCTTTTTCCTCCTGTTTTTCTGCCTGAACGCCACAATAGGAAACCCCAAGGAAGCCGCATGTGCCC
ATAAAGTATTCCACCTACGAAACGTGGCAGAAGAATGCGGCTCAGCGACTCCGGAGGGGCATCCCTGCC
ATCCTGAGGGCCAAAAAGTTTCGGAGGCAGGCGGAGAAGATGAAAGCTCAGGAGCAGGCCATCCTCCAC
CGGCCCCTGATTAGCCTTCCCAGCAAGCTGCCCGCCGTGTTGCACACCACAGACAATT -N17-

Appendix 2. *Igf2* ORF of *Girardinichthys multiradiatus* (San Matías el Grande population)

ATGGAGACCCAGCAAAGATCCGGACACCACTCACTTTGCCACACCTGCCGGACAACGGAGAGCTGCAGT
ATGAAGGTAACTGGAGCACAAATAGTTTCAGATTCCCCGTTTGAGCCGTGAGAAGGCTCAGGTAGAGCCG
ACTGGATAATTGTTGCGCAAAGGGGTACAGATAGATGCTTGTTGGCATCAGCTTTCTGCATTTTGAACAAG
CGGAATTTCTTGATTTGCATGCAGCAGAGGAAGTTCAGAATGCACAAACTGCAAAGGGAGACGCTTTGTG
TATTTATACCACGACAAGAAAGTAAAGAAATGAAAAGGTTATTTTTAATCTCCCCTTGATCTAAATAACGCG
CCGTTCGCCGGACACGGGCGCAGGAACAGCTGGTCGCCTTGCCGGCAGACAGGGGGCGGTAGGTCTGA
GGGTCATTGAGCTCACTCCGGGGTGAGCAAGGGGAAATTAAATGCTGAGTCTGTCTTTTTGGGGTAGTTA
ACTATCGAGCAGCCGGCCTTGCAACTTTCTGGGGTGGAGGGGGGAGTTGTGTAAGAATGAAAGCTTAGA
GATACTGGCGACTTTTTTCCTTCGGAATAAGCACTTCTCCCAGGTGCCAAACACATGACAGCAGCTGAAAA
AAATCGGGTCCTTGGTGTGTGTTTGAATGTGTTTATTTGATTTGATTTGACTCGCAGCGCACACTTTTTTCC
CCGAGTTACTAGGTCAGTGCGTGAAACAGCGTTTAAGACTTTAATCTCTTACATCTCGTACAAAACAAAAC
ATCTGGCTATTATTTGAGTTCTTTACATATAATTTTTTATTGTCATAATAATGGATCACAAGTCTAACATTTTT
CCGAATCTTATGCTTTAATTTGTGTGTTTCCCCCTGCAGGTCAAGAAGATGTGCGCGACCAGCCGTGCGC
TGCTCTTTGCGCTGACCCTCACGCTCTACGTTGTGGAAATGGCCTCGGCAGAGACGTTGTGTGGCGGAG
AGCTGGTGGATGCGCTGCAGTTTGTCTGCGAAGACAGAGGCTTCTATTTCAGTAGGTTTTTTTTTTTCAG
AGCTATGCAAGTTTCTCCAAAAACTAGCTGCGCAAATGTTGATTCGCCTACCTTTTTAATGTTATTCGCCCT
TTTCGCTCCCCTTTCCTCTCTGTTGTTTCACATCTGTTTGGATAGCTGAAACTGTCTGTGGCTGCGTGGTA
TACGTGTTTGCTATTTTTCTTTCTCACGCACTGACGCTCATGTAACACACACACAATCGCACACACGCCCG
TGCGCGCAAACGGCGTCAGAGAGAGAGAGAGAGAGAGAGAGAGAGGGAGAGAGAGGGAGATTGCAGCCAG
CTGTGCCTCTCCAGGTCTGCTCTGCTGAGTCTGCTCACAGCCTGCACCTCTCATTGCATATGAAAGGGCC
AGGCGGGCGAGCTGCAAAGGAACATGCACGACCCCGGCGTCACAATGGGCTGCTTGTTTATCAGTAAAG
TAATAGCACCCCAAGAGGCATTACCAGTCACCTCAGCACCAGTATTCCCAGGCCAGGAGACCACACTGAT
GAGCTGGGAGGAACGAGAGAAATAAAGAGAGAGAGAGAGAGATCGTTGTCAGGATGAGAGAGAAAAATA
AAATCAACATTTTATTATTTCTGATAGTTGCTTCCATAATATTGATGATGGGGTTGGCTGTATGGTTTTATGC
AGAGCATTGTCGTTAATCCTCAACTGGATAATCTTCTGTCTGCTTAGTCTGCGTTGCACGTGATATTCATAA
CTTTCCTGTTCTTCTGATTGCTCCCCGTTTTGCGTTTAAAAATACTGGCCACATTTAGGTATAGAGTGTTAA
AACTTACCACAAAATATTTTTTCATTTTTACTGGTGCTAAAATAATTGAAAATATTGATTATTGCAGTTATAAT
AATGTTATATGTTAGTGGTTGCCAAAGTTTTTGCTGGGATCCTACTCGGGGCATGAATCAGGAAGTGTAGG
GTCTTGAGATTCTCTTTGGAAACCAAAGTAAAACACAAACATGATCCTATAACTACCACATGATGTAAAGAG
CTGTTGTTATACATAGCTAAAAGCGATAAAGTGGTTGCTGATGTTATTTGCGTTATCATTATACTCCTATTTC
ATTGGCTTATTGTTAAAGCTTGCGTTCTAAATCTTACAGTTGGGATCACAGCTCTCTTCCACTAATTATTTG
TGGGTCAGAAGGTGGAAGGTTTAGGAACCAGTTATATGTAAAACATTTGTTTATTATTCTTTTGAGCGGTTA
ATAAATGGACTAAAGCAAAAATCTAGAATGAATTCAATGCTAGTATTCAATTGCTTTGGGACCTTCTTTGCT
GTTGTGCTCTATATAGAGTAACACAAATGTAACAAGCTGATAAAAGCTGGTTGATTAGTCAGGCTGTTCAG
GTAGCCAGCCTGCATTCTGCTGCTTAACAGACCAACTGCAGTTAGAGAATGGCATCCATAGTTTGTGCAA
CTGGAAATATTTCCAAACATCCAAATGTGACATCAGTGTAGCAGCCTTCTTTCAGCTATCTGACTGTCCTTG
TGCAGCTTTAAACCATAATGATTGTCTGACTAAAAATTTCAGTGGTTTTGAAACCGGAACTGTTCAAAACCA
CAGTATATGTTGATACTGGTAACCAGCCCACCCATGCCTAGCTGTGCTCCCGCTTCTCAATTGCCTTTTGA
CAAAGAGCAGAATCTTGTGTTTATCAAAGAAAATTTAGTCAATCAGTCCTCCCTCTCCCTGGATGACCCAG
TCTTCCTGTAATCAAATCTAGCCAAGCCTCATGTGAGACTTTTCTAACTTAGTGTGGGCTGCCAGGCTTCA
GACAAGGCCTTTATTGATCATGTGTAATGTTACCTTGTGGAAAACCCCTCACCTTTGCCTGCTATGTGTTA
CTTCTTCTCTTGTTTTGGTTTTACTGTTCTTACTTTCTGTTTCTACCCATTCTTTGCCAATTTGACAAAGCAA
ATGAGTTTTAGATTTAAATATTTAATTATTTTGAAGAATTTCTGAATGCATTCTCTGGTTTCTGGTCACTTTG
GACATGGTGTTTACTTTTAACACACTCATTTCGGGATGCCCAGATAGGCCTAATCAGATTATACCTTACAAC
CAAAAATATTTGTTGCTGTTCGTCGCAGATTTCCTTTTATTTGTCAATGTAATCCTGCAAAAACAACCGTAAT
TCGTTACTGTGGACAATGTGTGTTTTTTTGAAGGCCTCAACCTTCTATAACCTTTCTTACCGCTGTGTTTCT
CCTCAGGTAGGCCAACCAGCAGGGGTAACAACCGGCGGATCCAGACCCGTGGGATTGTAGAAGAGTGTT
GTTTCCGTAGCTGTGACCTCAACCTGCTGGAGCAATACTGTGCCAAACCCGCCAAGTCCGAGAGGGACG
TTTCGTCCTCCTCTATACAGGGCACGCCTGTTATGCCCACAACAAAGCAGGTGGGTCCAACCGGCAACCA
CAACACAAACTATAGTCCAGGTCTCTATCTGCCCTCTTATCTCACCTGAAACACAGAGTGCAGACTGAAAT
CCACACAAAAACAGGTTGATCTTGTTAAGTTCTCAGTGCTAAGCAGTTGTAAAATGCTGACCATGGTGGAA
AACATTAGTGCAATCACGGTACATTATATTGTAGAAATAGCAGCGTCAATAATCTCATGGTGAAGTAGCTG
AGTGGTGAACCGGAAAGAAACAGCATGTTTTGATTGTCTGATAATCAGTCAAAGAACAGCAAGCTGTCTCA
AAATATTCACATTCAAAAACACCACGTGTTTTTAGAAGAACTTTATTGGGAGGTGCCAGAAATGTGAATTAG
CTGAGACATCGTCAACACAATCTCTAAATTTTCTGCTAATAATATGCTAAAAAGAAGTATAAATGCTGCAGA

CTTAGAAAGCGATCTCTCACTATAACAGTAATTAAAAACAGGCTGCTGGGGAATTAGTTTGGATATGTCTC
CAGTTTGTCCCTGTGGGGAAAGTTGATGGTTGGAGGCTGCACTGAGGGCCAGCTGTTCTTGGGGCTATT
GAGCAGTTCTAGAGTGAATTAAGGGGGCTAAACACTCTTCAAGTGGCAGGACCAAAACAAGCTATTCACA
TGGAGCACTGGCTACTCACGCATAAATCCTACCTAGCTCAGCTGCCAGCATTCCCACTTGTCCCAAAACA
ACACCTACAACTGTTTGTCAGCATTAGCATAGATATTCTAAAATGAGTTTCCTTTGGTTGAGTGACAGCGTG
CAGTTAATATGTATATGAGGGGAGAATGGCCACCCTTCCTTTGTAATGGTATGCTAAAGAGGCCAAGCACT
TATTGACAGCTTCTCTCACTTGAGGAAACCAAAACAAGGGCGGGCTGGGGTCGAGATGAGGTTGTTTTGA
TGTTCACTGCTATAAGAATGAGATTTGATTTCTATTTTAGCAGCCCCAACAATGACCAAATGTTTTTGCGGA
ATAAGCAACACCTTACTCCATTGTAACGAGTTCTTGAACAGTAATCTTATCTCCGCTATTGAACTGACTTCC
TCTTCTCTCTTTTTCCTCCTGTTTTTCTGCCTGAACGCCACAATAGGAAACCCCAAGGAAGCCGCATGTGC
CCATAAAGTATTCCACCTACGAAACGTGGCAGAAGAATGCGGCTCAGCGACTCCGGAGGGGCATCCCTG
CCATCCTGAGGGCCAAAAAGTTTCGGAGGCAGGCGGAGAAGATGAAAGCTCAGGAGCAGGCCATCCTCC
ACCGGCCCCTGATTAGCCTTCCCAGCAAGCTGCCCGCCGTGTTGCACACCACAGACAATT-N17-

Appendix 3. *Igf2* ORF of *Girardinichthys viviparus*

ATGGAGACCCAGCAAAGATCCGGACACCACTCACTTTGCCACACCTGCCGGACAACGGAGAGCTGCAGG
ATGAAGGTAACTGGAGCACAAATAGTTTCAGATTCCCCGTTTGAGCCGTGAGAAGGCTCAGGTAGAGCTG
ACTGGATAATTGTTGCGCAAAGGGGTACAGATAGATGCTTGTTGGCATCAGCTTTCTGCATTTTGAACAAG
CGGAATTTCTTGATTTGCATGCAGCAGAGGAAGTTCAGAATGCACAAACTGCAAAGGGAGACGCTTTGTG
TATTTATACCACGACAAGAAAGTAAAGAAATGAAAAGGTTATTTTTAATCTCCCCTTGATCTAAATAACGCG
CCGTTCGCCGGACACGGGCGCAGGAACAGCTGGTCACCTTGCCGGCAGACAGGGGGCGGTAGGTCTGA
GGGTCATTGAGCTCACTCCGGGGTGAGCAAGGGGAAATTAAATGCTGAGTCTGTCTTTTTGGGGTAGTTA
ACTATCGAGCAGCCGGCCTTGCAACTTTCTGGGGTGGAGGGGGGAGTTGTGTAAGAGTGAAAGCTTAGA
GATACTGGCGACTTTTTTCCTTCGGAATAAGCATTCTCCCAGGAGCCAAACACATGACAGCAGCTGAAAAA
AATCGGGTGCTTGGTGTGTGTTTGAATGTGTTTATTTGATTTCATTTGACTCGCAGCGCACACTTTTTCCCC
CGAGTTACTAGGTCAGTGCGTGAAACAGCGTTTAAGACTTTAATCTCTTACATCTCGTACAAAACAAAACAT
CTGGCTATTATTTGAGTTCTTTACATATAATTTTTATTGTCATAATAATGGATCACAAGTCTAACAGTTTTCC
GAATGTTATGCTTTAATTTGTGTGTTTCCCCCTGCAGGTCAAGAAGATGTGCGCGACCAGCCGTGCGCTG
CTCTCTGCGCTGACCCTCACGCTCTACGTTGTGGAAATGGCCTCGGCAGAGACGTTGTGTGGTGGAGAG
CTGGTGGATGCGCTGCAGTTTGTCTGCGAAGACAGAGGCTTCTATTTCAGTAGGTTTTTTTTTTTCCAGA
GCTATGCAAGTTTCTCCAAAAACTAGCTGCGCAAATGTTGATTCGCCTACCTTTTTAATGTTATTCGCCCTT
TTCGCTCCCCTTTCCTCTCTGTTGTTTCACATCTGTTTGGATAGCTGAGACTGTCTGTGGCTGCGTGGTAT
ACGTGTTTGCTATTTTTCTTTCTCACGCACTGACGCCCATGCAACACACACACAATCGCACACACGCCCGT
GCGCGCAAACGGCGTCAGAGAGAGAGAAAGAGAGAGAGAGAGAGAGGGAGGGAGATTGCAGCCAGCTG
TGCCTCTCCAGGTCTGCTCTGCTGAGTCTGCTCACACAGCCTGCACCTCTCATTGCATATGAAAGGGCCA
GGCGGGCGAGCTGCAAAGGAACATGCACGACCCCGGCGTCACAATGGGCTGCTTGTTTATCAGTAAAGT
AATAGCACCCCAAGAGGCATTACCAGTCACCTCAGCACCAGTATTCCCAGGCCAGGAGACCACACTGATG
AGCTGGGAGGAACGAAAGAAATAAAGAGAGAGAGAAAGAGATCGTTGTCAGGATGAGAGAGAAAAATAAA
ATCAACATTTTATTATTTCTGATAGTTGCTTCCATTATATTGATGATGGGGTTGGCTGTATGGCTTTATGCA
GAGCATTGTCGTTAATCCTCAACTGGATAATCTTCTGTCTGCTTAGTCTGCGTTGCACGTGATATTCATAAC
TTTCCTGTTCTTCTGATTGCTCCCCGTTTTGCGTTTAAAAATACTGGCCACATTTAGGTATGGAGTGTTAAA
ACTTACCACATAATATTTTTTCATTTTTACTGGTGCTAAAATAATTGAAAATATTGATTATTGCAGTTATAATA
ATGTTATATGTTAGTGGTTGCCAAAGTTTTTGCTGGGATCCTACTCGGGGCATGAATCAGGAAGTGTAGG
GTCTTGAGATTCTCTTTGGAAACCAAAGTAAACCACAAACATGATCCTATAACTACCACATGATGTAAAGAG
CTGTTGTTATACATAGCTAAAAGCGATAAAGTGGTTGCTGATGTTATTTGTGTTATCATTATACTCCTATTTC
ATTGGCTTATTGTTAAAGCTTGCATTCTAAATCTTACAGTTGGGATCACAGCTCTCTTCCACTAATTATTTGT
GGGTCAGAAGGTGGAAGGTTTAGGAACCAGTTATATGTAAAACATTTGTTTATTATTCTTTTGAGCGGTTAA
TAAATTGACTAAAGCAAAAATCTAGAATGAATTCAATGCTAGTATTCAATTGCTTTGGGACCTTCTTTGCTG
TTGTGCTCTATATAGAGTAACACAAATGTAACAAGCTGATAAAAGCTGGTTGATTAGTCAGGCTGTTCAGG
TAGCCAGCCTGCATTCTGCTGCTTAACAGGCCAACTGCAGTTAGAGAATGGCATCCATAGTTTGTGCAACT
GGAAATATTTCCAAACATCCAAATGTGACATCAGTGTAGCAGCCTTCTTTCAGCTATCTGACCGTCCTTGT
GTAGCTTTAAACCATAATGATCGTCTGACTAAAAATTTCAGTGGTTTTGAAACCGGAACTGTTCAAAACCAC
AGTATATGTTGATACTGGTAACCAGCCCACCCATGCTTAGCTGTGCTCCCGCTTCTCAATTGCCTTTTGAC
AAAGAGCAGAATCTTGTGTTTATCAAAGAAAATTTAGTCAATCAGTCCTCCCTCTCCCTGGATGACCCAGT
CTTCCTGTAATCAAATCTAGCCAAGCCTCATGTGAGACTTTTCTAACTTAGTGTGGGCTGCCAGGCTTCAG
ACAAGGCCTTTATTGATCATGTGTAATGTTACCTTGTGAAAACCCCTCACCTTTGCCTGCTATGTGTTACTT
CTTCTCCTGTTTTGGTTTTACTGTTCTTACTTTCTGTTTCTACCCATTCTTTGCCAATTTGACAAAGCAAATG
AGTTTTAGATTTAAATATTTAATTATTTTGAAGAATTTCTGAATGCATTCTCTGGTTTCTGGTCACTTTGGAC
ATGGTGTTTACTTTTAACACACTCATTTCGGGATGCCCAGATAGGCCTAATCAGATTATCCCTTACAACCAA
AAATATTTGTTGCTGTTTGTCGCAGATTTCCTTTTATTTGTCAATGTAATCCTGCAAAAACAACCGTAATTCG
TTACTGTGGACAATGTGTGTTTTGTTGAAGGCCTCAACCTTCTATAACCTTTCTTACTGCTGTGTTTCTCCT
CAGGTAGGCCAACCAGCAGGGGTAACAACCGGCGGATCCAGACCCGTGGGATTGTTGAAGAGTGTTGTT
TCCGTAGCTGTGACCTCAACCTGCTGGAGCAATACTGTGCCAAACCCGCCAAGTCTGAGAGGGACGTTTC
GTCCTCCTCTATACCGGGCACGCCTGTTATGCCCACAACAAAGCAGGTGGGTCCAACCGGCAACCACAA
CACAAACTATAGTCCAGGTCTCTATCTGCCCTCTTATCTCACCTGAAACACAGAGTGCAGACTGAAATCCA
CACAAAAACAGGTTGATCTTGTTAAGTTCTCAGTGCTAAGCAGTTGTAAAATGCTGACCATGGTGGAAAAC
ATTAGTGCAGTCACAGTACATTATATTGTAGAAATAGCAGCGTCAATAATCTCAGGGTGAAGTAGCTGAGT
GGTGAACCGGAAAGAAACAGCATGTTTTGATTGTCTGATAATCAGTCAAAGAACAGCAAGCTGTCTCAAAA
TATTCACATTCAAAAACACCACGTGTTTTTAGAAGAACTTTATTGGGAGGTGCCAGAAATGTGAATTAGCTG
AGACATCATCAACACAATCTCTAAATTTTCTGCTAATAATATGCTAAAAATAAGTATAAATGCTGCAGACTTA
GAAAGCGATCTCTCACTATAACAGGAATTAAAAACAGGCTGCTGGGGAATTAGTTTGGATATGTCTCCAGT

TTGTCCCTGTGGGGAAAGTTGATGGTTGCAGGCTGCACTGAGGGCCAGCTGTTCTTGGGGCTATTGAGC
AGTTCTAGAGTGAATTAAGGGGGGTAAACACTCTTCAAGTGGCAGGACCAAAACAAGCTATTCACATGGA
GCACTGGCTACTCACGCATAAATCCTACCTAGCTCAGCTGCCAGCATTCCCACTTGTCCCAAAACAACGC
CTACAATTGTTTGTCAGCATTAGCATAGATATTCTAAAATGAGTTTCTGTTGGTTGAGTGACAGGGTGGAG
TTAATATGTATATGAGGGGAGAATGGCCACCCTTCCTTTGTAATGGTATGCTAAAGAGGCCAAGCACTTAT
TGACAGCTTCTCTCACTTGAGGAAACCAAAACAAGGGCGGGCTGGGGGCGAGATGAGGTTGTTTTGATGT
TCACTGCTATAAGAATGAGATTTGATTTCTATTTTAGCAGCCCCAACAATGACCAAATGTTTTTGCGGAATA
AGCAACGCCTTACTCCATTGTAACGAGTACTTGAACAGTAATCTTATCTCCGCTATTGAACTGACTTCCTCT
TCTCTCTTTTTCCTCCTGTTTTTCTGCCTGAACGCCACAATAGGAAGCCCCAAGGAAGCCGCATGTGCCCA
TAAAGTATCCCACCTACGAAACGTGGCAGAAGAAGGCGGCTGAGCGACTCCGGAGGGGCATCCCTGCCA
TCCTGAGGGCCAAAAAGTTTCGGAGGCAGGCGGAGAAGATGAAAGCTCAGGAGCAGGCCATCCTCCACC
GGCCCCTGATTAGCCTTCCCAGCAAGCTGCCCGCCGTGTTGCACACCACAGACAATT-N17-

Appendix 4. *Igf2* ORF of *Allotoca diazi*

ATGGAGACCCAGCAAAGATCCGGACACCACTCACTTTGCCACACCTGCCGGACAACGGAGAGCTGCAGG
ATGAAGGTAACTGGAGCACAAATAGTTTCAGATTCCCCGTTTGAGCCGTGAGAAGGCTCAGGCAGAGCTG
ACTGGATAATTGTTGCGCAAAGGGGTACAGATAGATGCTTGTTGGCATCAGCTTTCTGCATTTTGAACAAG
CGGAATTTCTTGATTTGTATGCAGCAGAGGAAGTTCAGGATGCTCAAACTGCAAAGGGAGACGCTTTGTG
TATTTATACCACAACAAAAAAGTAAAGAAATGAAAAGGTTATTTTTAATCTCCCCTTGATCTGAATAACGCG
CCGTTCGCCGGACACGGGCGCAGGAACAGCTGGTCGCCTTGCCGGCAGACAGGGGGCGGTAGGTCTGA
GGGTCATTGAGCTCACTCCGGGGTGAGCAAGGGGAAATTAAATGCTGAGTCTGTCTTTTTTGGGGTAGTT
AACTATCGAGCAGCCGGCCTTGCAACTTTCTGGGGTGGAGGGGGGAGTTGTGTAAGAGTGAACGCTTAG
AGATACTGGCGACTTTTTTCCTTCAGAATAAGCACTTCTCCCAGGTGCCAAACACATGACAGCAGCTGAAA
AAAATCGGGTGCTTGGTGTGTGTTTGAATGTGTTTATTTGATTTCATTTGACTCGCAGCGTACACTTTTTTC
CCCGAGTTACTAGGTCAGTGCGTGAAACAGCGTTTAAGACTTTAATCTCTTACATCTCGTACAAAACAAAA
CATCTGGCTATTATTTGAGTTCTTTATATATAACTTTTATTGCCATAATAATGGATGACAAGTCTAACAGTTT
TCCGAATCTCATGCTTTAATTTGTGTTTTTCCCCCTGCAGGTCAAGAAGATGTGCGTGACCAGCCGTGCGC
TGCTCTTTGCGCTGACCCTCACGCTCTACGTTGTGGAAATGGCCTCGGCAGAGACGTTGTGTGGGGGAG
AGCTGGTGGATGCGCTGCAGTTTGTCTGCGAAGACAGAGGCTTCTATTTCAGTAGGTTTTTTTCAGAGCTA
TGCAAGTTTCTCCAAAAACTAGCTGCGCAAATGTTGATTCGCCTACTTTTTTTTATGTTATTCGCCCTTCTC
GCTCCCTTTTCCTCTCTGTTGTTTCACATCTGTTTGGATAGCTGAGACTGTCTGTGGCTGCGTGGTATGCG
TGTTTGCTATTTTTCTTTCTCACGCACTGACGCCCATGCAACACACACACAATCGCACACACGCCCGTGCG
CGCAAACGGCGTCAGAGAGAGAGCGAGAGAGAGAGAGGGAGGGAGATTGCAGCCAGCTGTGCCTCTCC
AGGTCTGCTCTGCTGAGTCTGCTCACACAGCCTGCACCTCTCATTGCATATGAAAGGACCAGGCGGGCG
AGCTGCAAAGGAACATGCACGACCCCGGCGTCACAATGGGCTGCTTGTTTATCAGTAAAGTAATAGCACC
CCAAGAGGCATTACCAGTCACCTCAGCACCAGTATTCCCAGGCCAGGAGACCACACTGATTAGCTGGGA
GGAACGAAAGAAATAAAGAGAGAGAGATCGTTGTCAGGATGAGAGAGAAAAATAAAATCAACATGATTTTA
TTATTTCTGATAGTTGCTTCCATTGTATTGATGGTGGGGTTGGCTGTATGGCTTTATGCAGAGCATTGTCG
TTAATCCTCAACTGGATAATCTTCTTTCTGCTTAGTCTGCGTTGCACGTGATATTCATAACTTTCCTGTTCTC
CTGATTGCTCCCCGTTTTGCGTTTAAAAATACTGGCCACATTTAGGTATGGAGTGTTAAAACTTACCACATA
ATTGTTTTTAATTTTTACTGGTGCTAAAATAATTGAAAATATTGATTATTGCAGTTATAATAATGTTATATGTT
AGTGGTTCCCAAAGTTTTGGCTGGGATCCTACTCGGGGCATGAATCAGGAAGTGTAGGGTCTTGAGATTC
TCTTTGGAAACCAAAGTAAACCACAAACATGATCCTATAACTACCACATGATGTAAAGAGCTGTTGTTATAC
ATAGATAAAAGCGATAAAGTGGTTGCTGATGTTATTTGTGTTATCATTATACTCCTATCTCATTGGCTTATTG
TTAAAGCTTGCATTCTAAATCTTACAGTTGGGATTACAGCTCTCTTCCACTAATTATTTGTGGGTCAGAAGG
TGGAAGGTTTGGGAACCAGTTATATGTAAAACATTTATTTATTATTCTTTTGAGGGGTTAATAAATGGACTA
AAGCAAAAATCTAGAATGAATTCAATGCTAGTATTCAATTGCTTTGGGATCTTCTTTGCTGTTGTGCTCTAT
ATAGAGTAACACAAATATAACAAGCTGATAACAGCTGGTTGATTAGTCAGGCTGTTCAGGTAGCCAGCCTG
CATTCTGCTGCTTAACAGGCCAACTGCAGTTAGAGAATGGCATCCATAGTTTGTGCAACTGGAAATATTTC
CAAACATCCAAATGTGACATCAGTGTAGCAGCCTTCTTTCAGCTATCTGACCGTCCTTGTGCAGCTTTAAA
CCATAATGATCGTCTGACTAAAAATTTCAGTGGTTTTGAAACCGGAACTGTTCAAAACCACAGTATATGTTG
ATACTGGTAACCAGCCCACCCATGCCTAGCTGTGCTTCCGCTTCTCATTTTGCCTTTTGACAAAGAGCAGA
ATCTTGTGTTTATCCAAGAAACTTTAGACAATCAGTCCTCCCTCTCCCTGGATGACCCAGTCTTCCTGTAAT
CAAATCTAGCCAAGCCTCATGTGAGACTTTTTTAACTTAGTGTGGGCTGCCAGGCTTCAGACAAGGCCTTT
ATTGATCATGTGTAATGTTACCTTGTGAAAACCCCTCACCTTTGCCTGCTATGTGTTACTTCTTCTCCTGTT
TTGGTTTTACTGTTCTTACTTTCTGTCTCTACCCATTCTTTGCCAATTTGACAAAGCAAATGAGTTTTAGATT
TAAATATTTAATTATTCTGAAGAATTTCTGAATGCATTCTCTGGTTTCTAGTCACTTTGGACATGGTGTTTAC
TTCTAACACACTCATTTCGGGATGCCCAGATAGGCCTAATCAGATTATCCCTTACAACCAAAAATATTTGTT
GCTGTTCGTCGCAGATTTCCTTTTATTTGTCAATGTAATCCTGCAAAAACAACCGTAATTCGTTACTGTGGA
CAATGTGTGTTTTTTTTGAAGGCCTTAACCTTCTATCACCTTTCTTACCGCTGTGTTTCTCCTCAGGTAGGC
CAACCAGCAGGGGCAACAACCGGCGGATCCAGACCCGTGGGATTGTAGAAGAGTGTTGTTTCCGTAGCT
GTGACCTCAACCTGCTGGAGCAATACTGTGCCAAACGCGCCAAGTCCGAGAGGGACGTGTCGTCCTCCT
CTACACAGGGCACGTCTGTTATGCCCACACCAAAGCAGGTGGGTCCAACTGGCAACCACAACACAAAGTA
TAGTTAAGGTCTCTATCTGCCCTCTTATCTCACCTGAAACACAGAGTGCAGACTGAAATCCACACAAAAAC
AGGTTGATCTTGTTAAGTTCTCAGTGCTAAGCAGTTGTAAAATGCTGACCATGGTGGAAAACATTAGTGCA
GTCACAGTACATTATATTGTAGAAATTGCAGCGTCAATAATCTCAGGGTGCAGTAGCTGAGTGGTGAACCG
GAAAGAAACAGCATGTTTTGATTATCTGATAATCAATCAAAGAACAGCAAGCTGTCTCAAAATATTCCCATT
CAAAAACACCAAGTGTTTTTAGAAGAACTTTATTGGGAGGTGCCAGAAATGTGAATTAGCTGAGACATCGT
CAACACAATCTCTAAATTTTCTGCTAATAATATGCTAAAAAGAAGCATAAATGCTGCAGACTTAGAAAGCGA
TCTCTCACTATAACAGGAATTAAAAACAGGCTGCTGGGGATTTAGTTTGGACATGTCTCCAGTTTGTCCCT

GTGGGGACAGTTGATGGTTGCAGGCTGCACTGAGGGCCAGCTGTTCTTGGGGCTATTGAGCAGTTCTAG
AGTGAATTAAGGGGGGTAAACACTCTTCAAGTGGCAGGACCAAAACAAGCTATTCACATGGAGCACTGGC
TACTCACGCATAAATCCTACCTAGCTCAGCTGCCAGCATTCCCACTTGTCCCAAAACAACACCTACAACTG
TTTGTCAGCATTAGCATAGATATTCTAAAATGAGTTTCCTTTGGTTGAGTGACAGCGTGCAGTTAATATGTA
TATGAGGGGAGAATGGCCACCCTTCCTTTGTAATGGTATGCTAAAGAGGCCAAGCACTTATTGACAGCTT
CTCTCACTTGAGGAAACCAAAACAAGGGCGGGCTGGGGTCGAGATGAGGTTGTTTTGATGTTCACTGCTA
TAAGAATGAGATTTGATTTCTATTTTAGCAGCCCCAACAATGACCAAATGTTTTTGCGGAATAAGCAACACC
TTACTCCATTGTAACGAGTTCTTGAACAGTAATCTTATCTCCGCTATTGAACTGACTTCCTCTTCTCTCTTTT
TCCTCCTGTTTTTCTGCCTGAACGCCACAATAGGAAACCCCAAGGAAGCCGCATGTGCCCATAAAGTATTC
CACCTACGAAACGTGGCAGAAGAATGCGGCTCAGCGACTCCGGAGGGGCATCCCTGCCATCCTGAGGG
CCAAAAAGTTTCGGAGGCAGGCGGAGAAGATGAAAGCTCAGGAGCAGGTCATCCTCCACCGGCCCCTGA
TTAGCCTTCCCAGCAAGCTGCCCGCCGTGTTGCACACCACAGACAATT --N17—

Received: 7 December 2016 | Revised: 7 February 2017 | Accepted: 1 April 2017

DOI: 10.1002/ece3.3025

ORIGINAL RESEARCH

WILEY Ecology and Evolution Open Access

Asymmetric paternal effect on offspring size linked to parent-of-origin expression of an insulin-like growth factor

Yolitzi Saldivar Lemus[1] | Jean-Philippe Vielle-Calzada[2] | Michael G. Ritchie[3] | Constantino Macías Garcia[1]

[1]Instituto de Ecología, Universidad Nacional Autónoma de México, México City, Mexico

[2]UGA Laboratorio Nacional de Genómica para la Biodiversidad CINVESTAV Irapuato México, Irapuato, Mexico

[3]School of Biology, University of St. Andrews, St Andrews, UK

Correspondence
Constantino Macías Garcia, Instituto de Ecología, UNAM, México City, Mexico.
Email: maciasg@unam.mx

Funding information
Consejo Nacional de Ciencia y Tecnología, Grant/Award Number: 351769; PAPIIT, Grant/Award Number: IN216111; Howard Hughes International Research Program: 351769

Abstract

Sexual reproduction brings together reproductive partners whose long-term interests often differ, raising the possibility of conflict over their reproductive investment. Males that enhance maternal investment in their offspring gain fitness benefits, even if this compromises future reproductive investment by iteroparous females. When the conflict occurs at a genomic level, it may be uncovered by crossing divergent populations, as a mismatch in the coevolved patterns of paternal manipulation and maternal resistance may generate asymmetric embryonic growth. We report such an asymmetry in reciprocal crosses between populations of the fish *Girardinichthys multiradiatus*. We also show that a fragment of a gene which can influence embryonic growth (Insulin-Like Growth Factor 2; *igf2*) exhibits a parent-of-origin methylation pattern, where the maternally inherited *igf2* allele has much more 5′ cytosine methylation than the paternally inherited allele. Our findings suggest that male manipulation of maternal investment may have evolved in fish, while the parent-of-origin methylation pattern appears to be a potential candidate mechanism modulating this antagonistic coevolution process. However, disruption of other coadaptive processes cannot be ruled out, as these can lead to similar effects as conflict.

KEYWORDS

antagonistic coevolution, Goodeidae, matrotrophy, parental investment, sexual conflict, viviparous fish

1 | INTRODUCTION

Whenever individuals of different sexes interact, there is the potential for sexual conflict to occur, as the evolutionary interests of both individuals in relation to the outcome of the interaction are normally different (Parker, 1979, 2006). Conflict can arise in relation to current or future mating decisions and also in relation to how much each individual should invest in progeny (Trivers, 1972).

Even if offspring are cared for exclusively by members of one sex (hereafter, as is usually the case, the females), manipulation may still occur if females may be induced to invest preferentially in the brood of the current male, either through sensory manipulation (e.g., Burley, 1986) or by enhancing the ability of the offspring to extract resources from the mother. Viviparity induces an intimate physiological association between embryos and mother, which promotes offspring survival through regular direct provisioning and protection (Blackburn, 1999), while allowing females to adjust the amount and rate of resource delivery (Trexler & DeAngelis, 2003). Viviparous females must trade off current reproductive benefits against survival, future reproduction, and growth (Stearns, 1992). Males do not face the same trade-offs,

as they do not pay the costs but would enjoy greater benefits if the females they mate with increase their investment and produce bigger offspring or larger broods than they would otherwise (Crespi & Semeniuk, 2004; Griggio, Morosinotto, & Pilastro, 2009). Hence, antagonistic coevolution, with males manipulating female investment in offspring, may be expected in species where the amount of maternal investment can be modified after mating.

Sexual conflict can lead to evolutionary divergence (Arnqvist & Rowe, 2002; Chapman et al., 2003) as adaptations that are beneficial for the members of one sex prompt the evolution of countermeasures in the other to mitigate their negative effects (Arnqvist & Rowe, 2002). In the case of viviparous species, excessive male-induced increments of offspring provision may reduce the mother's lifetime breeding success. Thus, females that develop effective means to resist such manipulation would be favored, leading to a coevolutionary arms race (a form of intergenomic contest evolution, or ICE; Rice & Holland, 1997). Such a process may remain hidden if it leads to resolution of conflict, whereby male adaptations and female counter-adaptations come into balance (González-Forero, 2014). Therefore, a powerful method of finding evidence of such antagonism is to make crosses between members of independent populations and species, which are likely to differ in details of the antagonistic coevolution (Rowe, Cameron, & Day, 2003).

This was first observed in deer mice. When females of the monogamous *Peromyscus polionotus* mate with males of polygynous *P. maniculatus*, the size of the hybrids at birth is much larger than that of mice born to intraspecific matings, and they have 5–6 times heavier placentas than those of embryos from the reciprocal cross (Rogers & Dawson, 1970). Subsequent examples have been found in plants (reviewed by Alleman & Doctor, 2000), where several studies provide evidence that in plants with different mating systems, outcrossers can outperform self-pollinating parents (Brandvain & Haig, 2005), in insects, where the fecundity of honeybees has been shown to be influenced by epigenetic male manipulation (Oldroyd et al., 2014), and in fish, where Schrader and Travis (2008) found that the disruption of maternal-fetal coadaptation in crosses between populations of *Heterandria formosa* (a highly matrotrophic poeciliid species) results in differential embryo mortality linked to differences in maternal investment (Schrader, Fuller, & Travis, 2013; Schrader, Travis, & Fuller, 2011), and, again using interpopulation crosses, they demonstrated that embryos can influence maternal investment and that investment is traded versus fecundity (Schrader & Travis, 2009).

The best documented example of a mechanism of male epigenetic manipulation of female investment was the finding that the expression of the gene responsible for the synthesis of the insulin-like growth factor 2 (IGF2) and of its receptor (IGF2R) is epigenetically influenced in mouse embryos (DeChiara, Robertson, & Efstratiadis, 1991). IGF2 is a protein that promotes growth and cellular differentiation during development (Cohick & Clemmons, 1993), and in mammals, it also regulates the placental supply of nutrients and the demand of nutrients by the fetus (Constância et al., 2002). Excess IGF2 in the cell is captured and transported to the lysosomes for subsequent degradation by the cation-independent mannose-6-phosphate receptor, a membrane protein encoded by the gene *igf2r* (Kornfeld & Mellman, 1989), which plays an essential role in regulating normal fetal growth, circulating level of IGF2, and heart development (DeChiara et al., 1991; Lau et al., 1994). In therian mammals, these genes are expressed in a parent-of-origin manner. The paternal allele of *igf2* is translated while the maternal allele remains inactivated (DeChiara, Efstratiadis, & Robertson, 1990), and the opposite expression pattern is found in *igf2r*, which is maternally active and paternally silent in artiodactyls, rodents, and marsupials (although it is biallelically expressed in Scandentia, Dermoptera and Primates; Barlow et al., 1991; Killian et al., 2001a). Imprinting of these genes occurs in Therian mammals, but not in monotremes or birds (O'Neill et al., 2000; Killian et al., 2001b).

The *igf2* gene has been found in several fish species—including the Goodeidae (Poeciliidae, Lawton et al., 2005; Cyprinidae, Yuan et al., 2011), where it has been demonstrated to be under positive selection (O'Neill et al., 2007) and is expressed in their embryos. Additionally, matrotrophy, an advanced form of viviparity involving maternal provisioning of embryos through gestation, is present in at least 11 fish families, where it may have evolved independently (Wourms, Grove, & Lombardi, 1988). Theoretically, such viviparity has been considered to be potentially one of the main drivers of population divergence because of the close and particular physiological interactions between mother and embryo that may result in a conflict between them or between both parents over the level of maternal investment (Trivers, 1974; Zeh & Zeh, 2000).

One group of viviparous fish with advanced matrotrophy are the Mexican Goodeidae (Goodeinae, Lombardi & Wourms, 1985a,b). This is a clade of ca. 40 species distributed in 17 or 18 genera (Webb et al., 2004), a ratio of genera to species that suggests rapid speciation. This might be driven by the evolution of viviparity or possibly by the high prevalence of sexual selection, which is itself linked to the extreme sexual asymmetry in parental care that viviparity entails (Macías Garcia, 2014). Asymmetry in parental investment is particularly large in the Goodeinae, in which females nourish their embryos through unique specialized embryonic tissues known as trophotaeniae (Schindler, 2005) for 7–8 weeks (Macías-Garcia & Saborío, 2004), during which they grow up to 38,700% (Lombardi & Wourms, 1985a). Extended maternal provisioning and a specialized placenta-like structure make Goodeinae fish potentially good models for the study of antagonistic coevolution of parental allocation of resources to developing embryos, a possibility that has not previously been addressed. We looked for paternal effects on offspring development and size in crosses between populations, a pattern that could be consistent with antagonistic manipulation of offspring development.

2 | METHODS

2.1 | Study species

The Amarillo (Figure 1) is found in water bodies of the upper Lerma River basin, and in limited upland regions of the adjacent Balsas and Pánuco catchments (Gesundheit & Macias-Garcia, 2005). Males have much larger and colorful median fins than the females, who base their mate choice on these ornaments and on courtship performance (González Zuarth & Macías Garcia, 2006). There has been

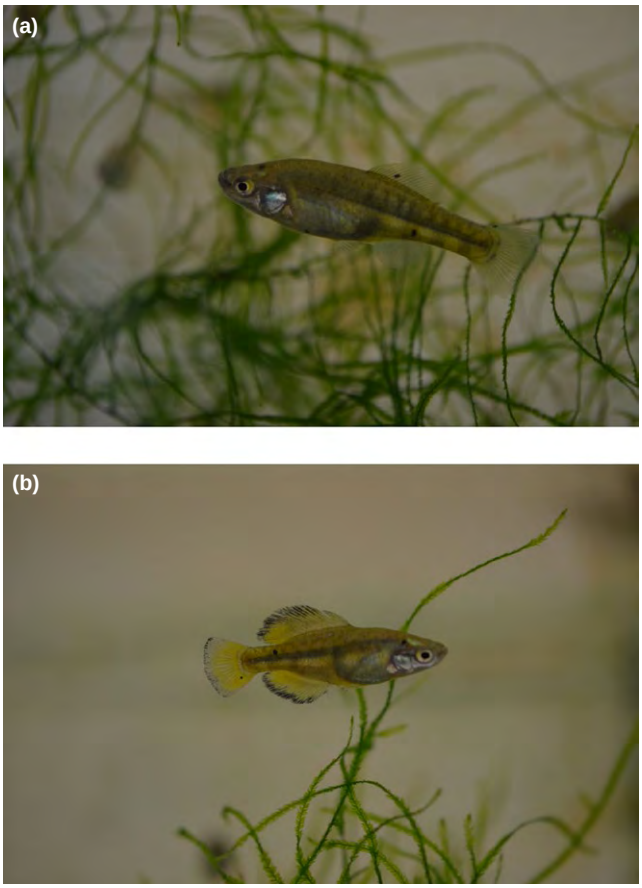


FIGURE 1 Photograph of a (a) female and (b) male of *G. multiradiatus* from San Matías el Grande population

rapid population divergence (Macías Garcia et al., 2012; Ritchie et al., 2007) and female mate choice often—but not always—leads to premating isolation between populations (González Zuarth & Macías Garcia, 2006; Macías Garcia et al., 2012). For this study, we selected the two populations that are most distant geographically and genetically; Zempoala (Z), a mountain population in the watershed between the southernmost reaches of the Lerma and the Balsas catchments, and San Matías (M), in the Balsas basin, at the northwestern corner of the Amarillo distribution (Macías Garcia et al., 2012). Genetic distance between these populations, based on microsatellite variation, is large (Macías Garcia et al., 2012). If offspring development is subject to some kind of parental antagonistic manipulation, we predicted that offspring size and weight would show paternal effects in crosses between populations. We also explored whether *igf2* shows evidence of a parental effect via parent-of-origin methylation patterns.

All methods were carried out in accordance with the guidelines for the treatment of animals in behavioral research and teaching published by Animal Behaviour (https://doi.org/10.1016/j.anbehav.2011.10.031). Fish were kept at the Instituto de Ecología, UNAM.

2.2 | Interpopulation crosses

Fish were collected under SAGARPA permit DGOPA/01262/040310.0716 and were promptly transported to aquaria at the Instituto de Ecología, UNAM in local water, stress coat, and antiseptics. They were maintained at a 12-hr day–night cycle, 21°C, and fed SeraVipan™ commercial fish flakes twice a day. New born fish were kept in 80-L population-specific aquaria until sex could be determined.

Between 60 and 125 days of age (100 ± 16 days; see De Gasperin & Macías Garcia, 2014), each fish was assigned to one of the following crosses (female–male): (1) M-M, (2) Z-Z, (3) M-Z, and (4) Z-M. Females were kept with the appropriate males either in one communal 80-L tank per cross (n = 49 females, or 62% of the final sample) or in smaller groups of one or two pairs, but at a comparable density within 20-L tanks (n = 30 females, 38% of the final sample). The distribution of females kept in either condition was similar for all crosses (x^2 = 1.2, df = 3, p = .75). Rearing condition, which was entered as a fixed factor in the analysis, had no effect on either brood or offspring size. Stress coat-treated gravid females were initially weighed once a week using an electronic scale (Ohaus Scout, SC2020) and then every two days as birth became imminent (usually in weeks 7 and 8). We did not measure male length because only about one-third of the broods (those born to pairs living in isolation) could be assigned to a particular sire.

Female body length (standard length) and width were measured from digital photographs taken the following day, once all offspring had been delivered, using UTHSCSA Image Tool freeware. Individual offspring were measured in the same way as their mothers, but their mass was obtained by weighing the entire brood and then dividing the value by the number of fish. Some females died during or shortly after giving birth but before all her measures were taken; therefore, we ended up with different sample sizes. We entered female length (SL) as a covariate in the analyses (female SL was highly and significantly correlated with female width; r = .93, $F_{(1,70)}$ = 418.7, p < .0001). We compared breeding performance and brood attributes using mixed models in which each brood was used only once on each analysis (brood size, mean offspring mass, and the ratio of brood mass/female mass before parturition (reproductive allocation; RA; Abrahamson & Gadgil, 1973). Individual offspring SL and width were nested within brood. All our mixed models included female identity as a random factor, female SL and rearing environment as covariates, and female population of origin as one fixed factor; they also included male population of origin and the interaction between male and female population, as these two effects would be indicative of offspring genotype influencing female parental investment (Reznick, 1981; Schrader & Travis, 2009). Reported post hoc probabilities are corrected (Bonferroni) for multiple comparisons. All analyses were performed using NCSS 2007 v. 7.1.21.

2.3 | Parent-of-origin *igf2* expression

A ≈5-kb fragment of *igf2* was cloned and sequenced using primers adapted from the published sequence of *Ilyodon ameca* (GenBank Accession number DQ337453.1) (see Appendix S1 for details on *igf2* sequencing and SNPs analysis), and screened for SNPs. We used a SNP located in the coding region of fish from Zempoala to evaluate parent-of-origin expression of *igf2*. First, we generated several breeding groups, always made of one Z–Z pair, and in some cases an additional female from either Huapango (in the vicinity of San Matías) or

Tonatiahua (in the Zempoala lakes National Park), as females from San Matías were temporarily unavailable. Fish within an interpopulation pair were raised together to overcome preferences for intrapopulation partners (De Gasperin & Macías Garcia, 2014). Fish were kept under standardized conditions (see Appendix S1), and each resulting pregnant female and her entire brood were sacrificed around the 7th week of pregnancy, when we collected a fin clipping from the sire and stored the tissues either in absolute ethanol or in RNAlater.

2.3.1 | Genotyping of families

The *igf2* gene of teleosts is typically composed of four exons and three introns (Juhua et al., 2010). We screened for SNPs from exon 2 (Figure 2) of 22 breeding pairs (36 individuals, as five males were shared by two females and one male by three females). Primers (see Appendix S1) amplified a product of 443 nucleotides that contained exon 2 in its entirety, plus some segments of the adjacent introns.

Genomic DNA was extracted with a homemade protocol (see Appendix S1 for details). The PCR reaction system contained 10 μl of GoTaq Green Master Mix (Promega), 6 μl Milli Q water, 1 μl DMSO, 1 μl I2_F_P (20 pmol/μl), 1 μl I2_R_P (20 pmol/μl), 1 μl DNA, and was exposed to 30 PCR cycles of 95°C for 5 min, 94°C for 30 s, 59°C for 30 s, 72°C for 30 s followed by 10 min at 72°C. PCR products were cloned using TOPO TA Cloning kit (Invitrogen) for electrocompetent cells (TOP10 Electrocomp). Plasmid DNA was extracted following alkaline lysis protocol by Sambrook, Fritsch, and Maniatis (1989). A minimum of 10 clones per individual were sent for sequencing, and sequences were analyzed with BioEdit Sequence Alignment Editor. We genotyped the offspring of families in which we could track the parental alleles (i.e., parents were not homozygous for the same SNP) and screened the brood for heterozygous embryos, as before.

2.3.2 | Assessing gene expression through RT-PCR

Total mRNA of heterozygous offspring was isolated using TRIzol (Invitrogen) and was then reverse transcribed using SuperScript II Reverse Transcriptase and oligodT primers, according to the manufacturer's protocol (Invitrogen). The cDNA was employed as a template for PCR amplification using specific primers. To distinguish the size of the amplified cDNA product from genomic fragments that could be amplified after inefficient DNAse digestion, forward and reverse primers were anchored in exons 1 and 2, respectively. We cloned the fragment as before, analyzed a minimum of 50 clones per individual, and determined which allelic variant (parental allele) had been recovered.

2.4 | Bisulfite sequencing

Parent-of-origin expression effects often occur by genomic DNA methylation, involving the addition of a methyl group to cytosine residues of the dinucleotide CpG (Hendrich & Tweedie, 2003).These can be revealed by treating genomic DNA with bisulfite, which converts cytosine residues to uracil (translated into thymine during sequencing). To determine whether the asymmetric effects on offspring size could be influenced by parent-of-origin effects in the methylation state of *igf2*, we took advantage of a heterozygous C/T embryo (P21-3) that inherited a T allele from its mother and a C allele from its father, and of a heterozygous C/T adult female (P11-F)—although here we did not know the parental origin of each allele—and analyzed the pattern of 5′ cytosine methylation in a 443-bp fragment that spanned the SNP site by treating genomic DNA with bisulfite before PCR amplification and cloning.

Bisulfite sequencing was performed as reported in Lim et al. (2015) with minor modifications using DNA from the two individuals mentionned above. Samples of 500 ng of genomic DNA (obtained as above) were bisulfite converted using EZ DNA methylation-direct kit (Zymo Research), eluted in 30 μl elution buffer, and 1 μl of each aliquot was PCR amplified using primers forward: Forward1 and Forward2 and reverse: Reverse1 and Reverse2 (designed as above; see Appendix S1: Table S2) (95°C for 5 min, 20 cycles of 94°C for 30 s, 59°C for 30 s, 72°C for 30 s each, 72°C for 10 min.). PCR products were gel purified, cloned into pDRIVE cloning vector using Qiagen PCR cloning kit (Qiagen, Valencia, CA), and transformed into DH10B cells before sequencing.

3 | RESULTS

3.1 | Interpopulation crosses

F1 females from Z were smaller than from M ($t = 4.57$, $df = 70$, $p < .0001$; Table 1) but gave birth to larger broods than M females (Bonferroni $F_{(1,66.0)} = 6.30$, $p = .01$; Table 1). Furthermore, Z females did not produce smaller offspring than M females when mated with males of their own population (Z-Z vs. M-M; Bonferroni $F_{(1,67.1)} = 0.93$,

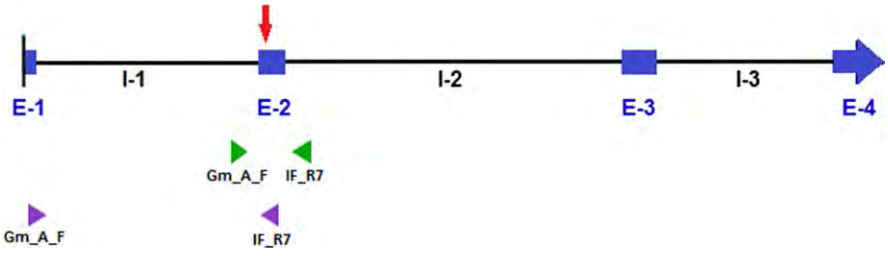


FIGURE 2 Scheme of *igf2* of *G. multiradiatus*. Blue boxes represent exons, continuous lines introns, and the red arrow shows the approximate location of the selected SNP. Green arrowheads represent the binding site of the primers used for genotyping, and purple arrowheads show the binding site of the primers used for RT-PCRs.

TABLE 1 Size and fecundity of F1 females

	Cross											
	M–M			M–Z			Z–M			Z–Z		
Variable	*X*	*SD*	*N*	*X*	*SD*	*N*	*X*	*SD*	*N*	*X*	*SD*	*N*
Mother												
SL (mm)	34.05	5.51	15	34.79	3.68	11	30.32	3.83	17	29.05	4.18	29
W (mm)	8.82	1.6	15	9.35	1.27	11	7.68	1.35	17	7.27	1.11	29
Mass (g)	0.56	0.21	16	0.54	0.16	11	0.36	1.36	17	0.32	0.17	29
Brood size	6.1	4.46	19	5.54	3.38	13	5.72	3.91	18	6.59	3.12	30
RA	0.14	0.08	16	0.15	0.07	11	0.17	0.08	17	0.14	0.06	29
Mean offspring												
SL (mm)	11.2	1.28	16	11.73	0.78	11	11.36	1.13	17	10.3	1.11	28
W (mm)	2.55	0.5	16	2.69	0.29	11	2.56	0.34	17	2.25	0.36	28
Mass (g)	0.17	0.006	16	0.18	0.005	11	0.15	0.008	17	0.1	0.005	29

Reproductive allocation (RA) is the ratio of total brood mass/brood + mother mass (SL, standard length; W, width).

p = 1.0; Figure 3a), and when mated with M males, they gave birth to larger offspring than to those produced by Z females mated with Z males (Bonferroni $F_{(1,63.0)}$ = 9.83, p = .02). Also, we detected a significant interaction between male and female population of origin on offspring size ($F_{(1,64.4)}$ = 7.45, p = .008) as well as a significant effect of the cross ($F_{(3,64.6)}$ = 3.7, p = .02). We observed a similar pattern with offspring width, with no effect of female population ($F_{(1,65.2)}$ = 0.94, p = .34), and a significant male X female interaction ($F_{(1,65.2)}$ = 4.51, p = .04); offspring of Z-M were wider than those from Z-Z crosses (Bonferroni $F_{(1,63.5)}$ = 7.14, p = .02; Figure 3b). Neither length nor width of offspring from M females differs between crosses (length, Bonferroni $F_{(1,65.6)}$ = 1.17, p = .57; width, Bonferroni's $F_{(1,66.4)}$ = 0.32, p = 1; see Appendix S1: Tables S3 and S4).

Neonates produced by M females were heavier than those produced by the smaller Z females ($F_{(1,66.0)}$ = 5.15, p = .03; Table 1; Figure 3c). Weight significantly covaried with maternal length ($F_{(1,66.0)}$ = 6.21, p = .015). As above, Z females mated with M males produced heavier offspring than their controls ($F_{(1,66.0)}$ = 7.17, p = .02), although the male X female interaction was not significant.

Zempoala females appear to allocate more resources to offspring production than females from San Matías (although the difference fell short of significance; $F_{(1,52.0)}$ = 3.19, p = .08; see Appendix S1: Fig. S2a) but this apparent difference was not related to the population of origin of the male sire (male × female interaction, $F_{(1,52)}$ = 1.12, p = .29); thus, the interaction between paternal and maternal population of origin on offspring size was not due to a male influence on the female RA.

3.2 | Parent-of-origin *igf2* expression

3.2.1 | Genotyping of families

We only found one SNP (C/T) sufficiently frequent to be used as a marker of parent-of-origin expression of *igf2*, yet in spite of extensive crosses (n = 22 pairs), only three heterozygous offspring were obtained. When cloning the gene from two of these, only the paternal allele was recovered. Although suggestive, our assessment of parent-of-origin expression of *igf2* is, consequently, inconclusive given the scarcity of heterozygous fish (see Appendix S1 for details).

3.3 | Bisulfite sequencing

Thirty-eight independent fragments of *igf2* from P21-3 were sequenced: 20 corresponded to the maternally and 18 to the paternally inherited *igf2* copy (X^2 = 0.105, df = 1, p > .05). Strikingly, 5′-methylcytosines in a CpG context were only prevalent (i.e., present in >50% of the clones) in sequences representing a maternally inherited *igf2* copy and were virtually absent from copies that were paternally inherited (Figure 4a). Additional cytosines present in non-CpG positions were also frequently methylated in the maternally inherited *igf2* copy, contributing to a highly contrasting methylation pattern that correlates with the monoallelic expression of *igf2* during embryogenesis.

Seventeen independent sequences from adult female P11-F were obtained; the eight belonging to one allele were hypomethylated, and the nine sequences of the other allele were hypermethylated (Figure 4b). As with P21-3, these segregations are not different from 1:1 (X^2 = 0.06, df = 1, p > .05), indicating that the cytosine residue present at position 225 of the amplified fragment (corresponding to the P21-3 paternally inherited *igf2* copy) is not affected by the bisulfite treatment, allowing for a comparison of the methylation pattern among both alleles.

4 | DISCUSSION

Here, we demonstrate an interaction between paternal and maternal origin in the size attained at birth by *G. multiradiatus* offspring. This is not the consequence of population differences in female size, nor,

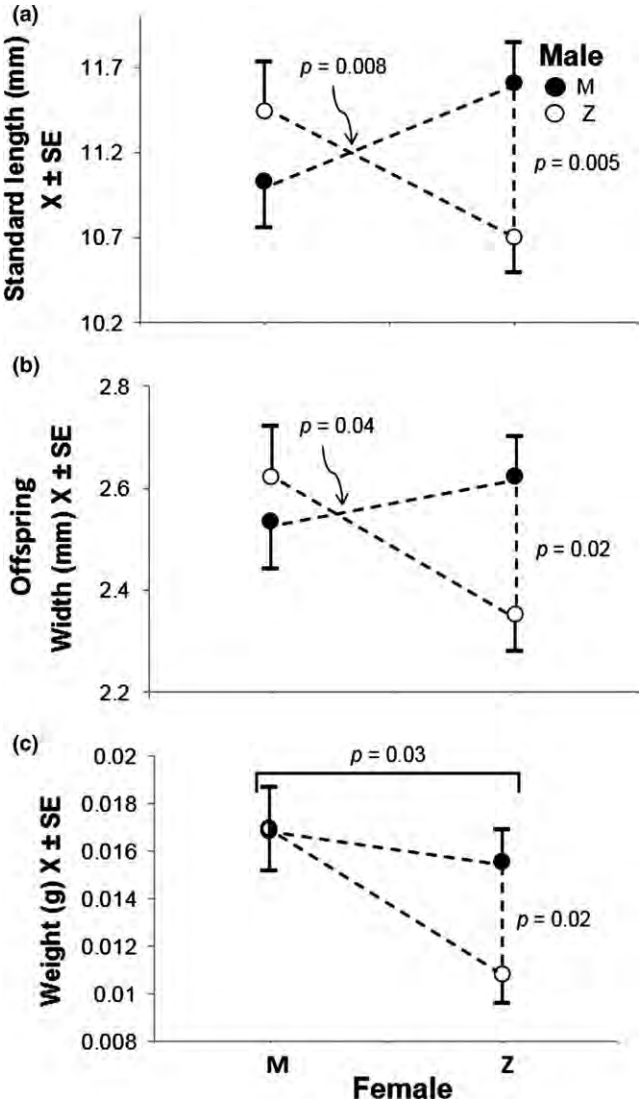


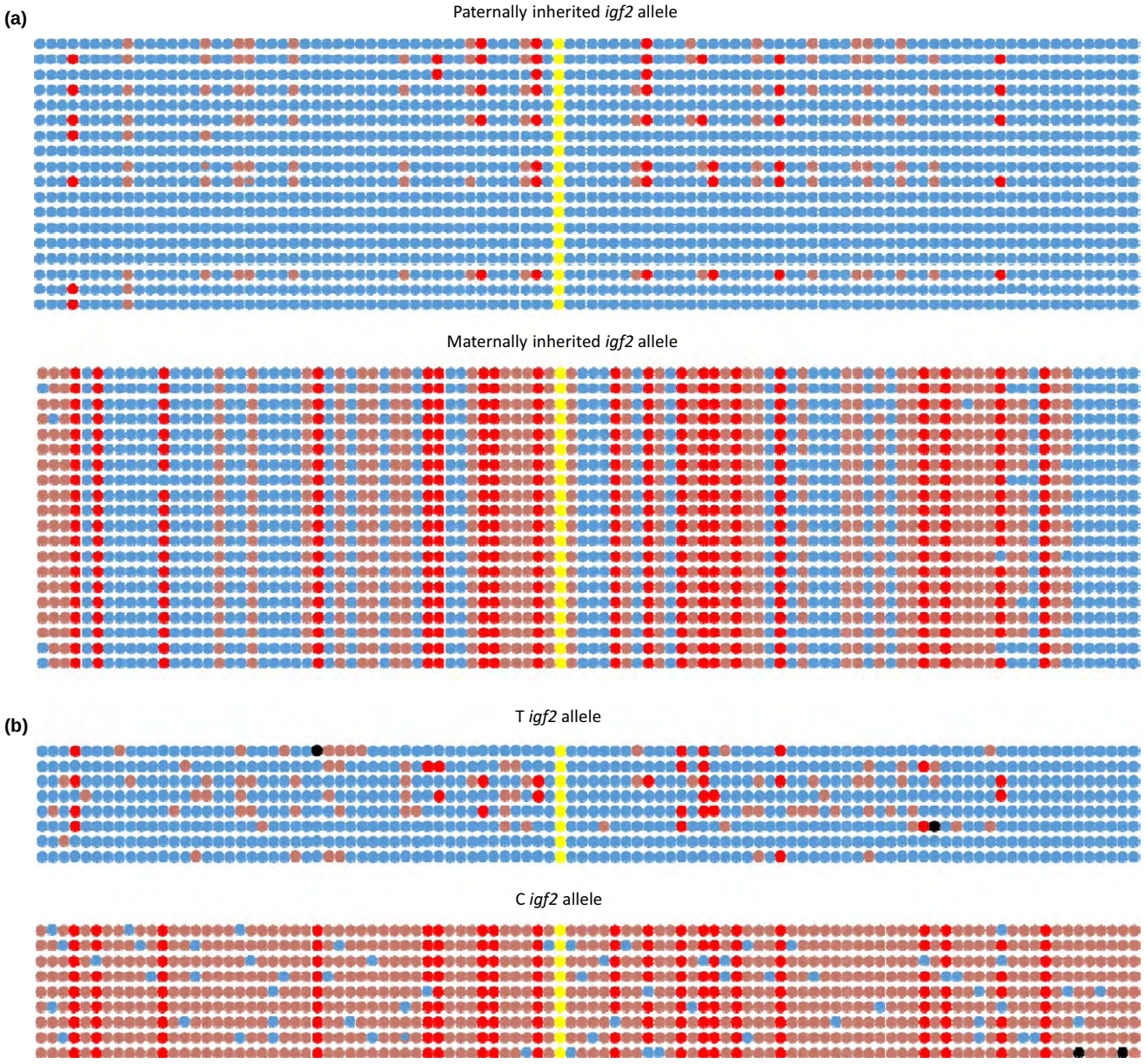
FIGURE 3 (a) Length (SL), (b) width, and (c) weight of the offspring from intra- and interpopulation crosses of adult *G. multiradiatus*. Significant interactions between paternal and maternal (*X*-axis) origin seen in (a) and (b) are predicted when there is sexual conflict over parental provisioning of embryos. Graphs based on adjusted means to discount the effect of correlated female size

apparently, of major differences in reproductive allocation (but see Appendix S1). We also found differences in how resources transferred to their embryos are used in both populations. While offspring size was similar, the smaller females from Zempoala produced more, but lighter, newborn fish than their San Matías counterparts. These patterns are influenced by sire, because Zempoala females, when mated with a male from San Matías, resulted in larger, wider, and heavier offspring than mating with a Zempoala male (Figure 3), further suggesting that the male origin influences offspring growth in this matrotrophic fish through an interaction between the maternal and paternal contributions. We did not find evidence that brood number was influenced by the males, which may signify that females have a somewhat fixed amount of resources to invest or a set number of ova to fertilize in each brood (see Appendix S1: Fig. S2b).

The size of offspring from matings between populations can depart from additive expectations for several reasons. In positive heterosis (hybrid vigor, or simply "heterosis"; Shull, 1908), offspring from both reciprocal crosses would be expected to be similarly larger (or healthier, or fitter) than offspring form the parental populations (e.g., Shikano, Nakadate, & Fujio, 2000), whereas outbreeding depression would cause smaller or less fit offspring in both interpopulation crosses. These effects are expected to be symmetrical, due to either the amelioration of mutational load (Keller & Waller, 2002) or the breakdown of co-adapted genomes (Templeton et al., 1986) and are thus unlikely explanations for the phenotypic effects seen here (but we note that M-Z hybrids were somewhat, but nonsignificantly, larger than M-M offspring). Furthermore, disruption of coadapted complexes in F1 hybrids is usually only seen in one sex (Haldane, 1922), and although we do not have data on the sex of the newborn, we found no male X female effect in the coefficient of variation of offspring size ($F_{(1,66)} = 0.21$, $p = .65$), as would have been expected if Haldane's rule was occurring.

Differences between parental and hybrid phenotypes can also be the consequence of maternal effects if, for instance, females perceive the males from the alternative populations to be more attractive than those from their own, and preferentially invest in offspring of attractive males (Burley, 1988; Gil et al., 1999). This is unlikely to explain our results as females from both localities are reluctant to mate with males from the other population (González Zuarth & Macías Garcia, 2006) unless they are raised together from an early age (De Gasperin & Macías Garcia, 2014). Maternal effects are also an unlikely explanation because only the offspring of Z-M crosses, and not those from M-Z, were larger than their controls (Figure 3a).

Breakdown of genetic coadaptations can result in phenotypic effects in interpopulation crosses such as those described here. Genes will have coevolved to function properly in the context of other genes involved in the same processes, giving raise to coadapted clusters of genes that may differ between populations (Wolf, 2013). Crosses between populations may break down such coadapted clusters and generate a diversity of unpredictable phenotypic patterns. Similarly, the details of the necessary coadaptation between mother and embryos may vary between populations and may also be disrupted by interpopulation crosses (Wolf & Brodie, 2009). Disruption of gene coadaptation through outcrossing may also lead to genes been silenced (Ortíz-Barrientos, Counterman, & Noor, 2007), which might lead to monoallelic expression or to the disruption of genomic imprinting (Wolf, Oakey, & Feil, 2014). Our results are also consistent with expectations derived from sexual conflict (Parker, 1979, 2006). Goodeid matrotrophic viviparity involves a massive, protracted transfer of nutrients to the embryos (Lombardi & Wourms, 1985a,b) that can be co-opted by males. There is no evidence suggesting that males can influence female investment through sensory stimulation during courtship, but we show evidence that *igf2*, a gene whose overexpression may influence embryonic growth, has a parent-specific methylation pattern, which suggest a possibly epigenetic parental effect. At present, we cannot distinguish between the co-adaptation and the conflict hypotheses (see Schrader et al., 2013).



5'GAGTTACCAGGTCAGTGCGTGAAACAGCGTTTAAGACTTTAATCTCTTACATCTCGTACAAAACAAAACATCTGGCTATTATTTGAGT
TCTTTACATATAATTTTTATTGTCATAATAATGGATCACAAGTCTAACATTTTTCCGAATCTTATGCTTTAATTTGTGTGTTTCCCCCTGCAG
GTCAAGAAGATGTGCGCGACCAGCCGTGCGCTGCTCTTTGCGCTGACCCTCACGCTCTACGTTGTGGAAATGGCCTCGGCAGAGACG
TTGTGTGGCGGAGAGCTGGTGGATGCGCTGCAGTTTGTCTGCGAAGACAGAGGCTTCTATTTCAGTAGGTTTTTTTTTTTTCAGAGCT
ATGCAAGTTTCTCCAAAAACTAGCTGCGCAAATGTTGATTCGCCTACCTTTTTAATGTTATTCGCCCTTTTCGCTCCCCTTTCCTCT3'

FIGURE 4 Parent-of-origin effects in genomic DNA methylation at the *igf2* gene. 5′–3′ linear representation of cytosines present in a 443-bp genomic fragment spanning an informative SNP (highlighted in yellow) that allow distinction between maternally and paternally inherited IGF2 gene copies in a) a heterozygous offspring (P21-3) and b) a heterozygous female (P11-F); 5′ methylated cytosines in a CpG context are represented by dark red dots, 5′ methylated cytosines in a different context are shown as light red dots, unmethylated cytosines are indicated as blue dots, and cytosines of undetermined methylation status are indicated as black dots. The lineal sequence of the fragment is shown below the graphic depiction of methylation. The cytosines are highlighted in light blue, and the nucleotide of the SNP (C/T) is highlighted in yellow

The *igf2* gene encodes insulin-like growth factor 2 (IGF2) which plays an important role in embryonic development. It is involved in nutrient exchange between mother and embryo (Constância et al., 2002; Reik et al., 2003) and can, therefore, affect the amount of nutrients transferred to the developing offspring. An analysis of non-synonymous mutations in the mRNA of *igf2* has shown this gene to be under positive selection in several placental cyprinodontiformes (O'Neill et al., 2007), implying that it is involved in the development

of matrotrophic fish embryos. O'Neill et al. (O'Neill et al., 2007) inferred that evidence of sustained directional selection on the coding sequence of this gene in matrotrophic cyprinodontiformes amounts to evidence of parent–offspring conflict driving *igf2* evolution. This is plausible, but the argument cannot be compelling unless it is also shown that either 1) an antagonistic gene (e.g., the *igf2r*) has experienced a comparable evolutionary divergence, or 2) that the expression of *igf2* in the embryos follows a parent-of-origin pattern (i.e., that there is a bias in embryos to express the paternal allele). We only demonstrated a parent-of-origin methylation pattern in the developing embryos, but these data, together with (1) the evidence of *igf2* being expressed in fish embryos (Lawton et al., 2005; Yuan et al., 2011), (2) a parent-specific methylation pattern in gametes of an oviparous fish (suggesting that the foundations of genomic imprinting also exist in teleost fish; Xie et al., 2009), and (3) the evolution in fish of the manose-6-phosphate receptor into an insulin-like growth factor 2 receptor (*igf2r*) with a role on *igf2* degradation (Nolan et al., 2006) that has a similar structure and affinity for IGF2 to that of the mammalian gene (Méndez et al., 2001), suggests that the possibility of genetic imprinting in this group of viviparous vertebrates should be investigated.

Paternal manipulation in developing offspring may be countered by maternal adaptations to mitigate its effects. If this antagonistic coevolution is not completely matched in isolated populations, asymmetric embryonic growth of the type that we detected in the interpopulation crosses may occur (although we did not find a substantial decrease in embryo size in M-Z broods, which would provide evidence of co-evolved female resistance to any manipulation by the males; see Moore & Haig, 1991).

A parent-of-origin *igf2* methylation pattern in *G. multiradiatus* may be the consequence of several processes, including epigenetic regulation as that seen in mammals (Lawton et al., 2008; Murrell, Heeson, & Reik, 2004). Our data indicate that *G. multiradiatus* females from different populations produce offspring of different size, but do not modify the number of offspring per brood, when mated with allopatric males. This could happen if maternal factors were differentially at play and is also consistent with male manipulation of female reproductive allotment; and experimental manipulation is required to tease these possibilities apart.

The sexually antagonistic IGF system is only known to occur in mammals, but its constitutive elements are found in fish, raising the possibility that it evolved independently in mammals and teleosts, or that it was present in the ancestors of the two lineages diverged. Previous efforts to demonstrate imprinting of *igf2* in placental Poeciliid species have been unsuccessful (Lawton et al., 2005); yet, we found evidence that suggest parent-of-origin gene expression in the Goodeidae (which are also cyprinodontids). Some attributes that may favor the evolution of a genetic antagonistic coevolution mediated by IGF2 in the Goodeidae include enforceable female mate choice. This may be linked to the fact that goodeid embryos' dry weight can increase up to 38,700% (*Zoogoneticus quitzeoensis;* Wourms et al., 1988; Hollenberg & Wourms, 1995), whereas placental poeciliid embryos achieve at most 11,700% (*P. retropinna*; Wourms 1981). Such greater mass increase takes place during a gestation period that lasts about 8 weeks; twice as much that of poeciliids. We think that the massive reproductive allocation of goodeid females, together with the existence of a trophotaenial placenta (a fetal structure involved in the capture and transport of nutrients from the ovarian lumen/walls to the embryonic gut; Lombardi & Wourms, 1985a,b), provides both the opportunity and the physiological conditions in which *igf2* can influence maternal investment.

The breeding system of the Amarillo (*Girardinichthys multiradiatus*) fits the conditions stipulated by Wilkins and Haig (2003) as potential promoters of genomic imprinting: (1) Broods can be sired by more than one male (Macías-Garcia & Saborío, 2004), (2) females bear the bulk of the reproductive costs (e.g., Lombardi & Wourms, 1985a,b), and (3) their allocation of resources can be influenced by genes that are expressed in the embryos (e.g., *igf2*; see O'Neill et al., 2007); therefore, further research on the possibility of genomic imprinting of *igf2* of this fish is needed.

ACKNOWLEDGMENTS

We thank T. Sneddon, J. Graves, G. Smith, and C. Álvarez for technical support. Edgar Ávila-Luna helped with the fish husbandry and S. Ramirez Esparza performed the bisulfite experiment. Research was supported by PAPIIT grant IN216111 (CMG), CONACyT PhD scholarship 351769 (YSL), and the Howard Hughes International Research Program (JPVC). This work was conducted as partial fulfillment of YSL Ph.D. thesis within the Programa de Doctorado en Ciencias Biomédicas (Universidad Nacional Autónoma de México) under the supervision of CMG.

CONFLICT OF INTERESTS

All authors declare that we do not have any competing financial interests.

AUTHOR CONTRIBUTIONS

YSL carried out most of the experiments and analyses and wrote the first draft of the manuscript in conjunction with CMG. J-PV-C provided logistical, technical, and financial support. MGR provided logistical and technical support. CMG provided logistical support and wrote the first draft of the manuscript in conjunction with YSL. All authors contributed to the design of the experiments, the interpretation of the results, and the edition of the manuscript.

REFERENCES

Abrahamson, W. G., & Gadgil, M. (1973). Growth form and reproductive effort in goldenrods (Solidago, Compositae). *The American Naturalist*, *107*(957), 651–661.

Alleman, M., & Doctor, J. (2000). Genomic imprinting in plants: Observations and evolutionary implications. *Plant Molecular Biology*, *43*, 147–161.

Arnqvist, G., & Rowe, L. (2002). Antagonistic coevolution between the sexes in a group of insects. *Nature*, *415*, 787–789.

Barlow, D. P., Stöger, R., Herrmann, B. G., Saito, K., & Schweifer, N. (1991). The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature*, *349*, 84–87.

Blackburn, D. G. (1999). Viviparity and oviparity: Evolution and reproductive strategies. *Encyclopedia of Reproduction*, *4*, 994–1003.

Brandvain, Y., & Haig, D. (2005). Divergent mating systems and parental conflict as a barrier to hybridization in flowering plants. *The American naturalist*, *166*(3), 330–338.

Burley, N. (1986). Comparison of the band-colour preferences of two species of estrildid finches. *Animal Behaviour*, *34*(6), 1732–1741.

Burley, N. (1988). The differential-allocation hypothesis: An experimental test. *American Society of Naturalists*, *132*(5), 611–628.

Chapman, T., et al. (2003). Sexual conflict. *Trends in Ecology & Evolution*, *18*(1), 41–48.

Cohick, W. S., & Clemmons, D. R. (1993). The insulin-like growth factors. *Annual Review of Physiology*, *55*(1), 131–153.

Constância, M., Hemberger, M., Hughes, J., Dean, W., Ferguson-Smith, A., Fundele, R., … Reik, W. (2002). Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature*, *417*, 945–948.

Crespi, B., & Semeniuk, C. (2004). Parent-offspring conflict in the evolution of vertebrate reproductive mode. *American Naturalist*, *163*(5), 635–653.

De Gasperin, O., & Macías Garcia, C. (2014). Congenital predispositions and early social experience determine the courtship patterns of males of the Amarillo fish. *Behavioral Ecology and Sociobiology*, *68*(4), 639–648.

DeChiara, T. M., Efstratiadis, A., & Robertson, E. J. (1990). A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature*, *345*, 78–80.

DeChiara, T. M., Robertson, E. J., & Efstratiadis, A. (1991). Parental imprinting of the mouse insulin-like growth factor II gene. *Cell*, *64*(4), 849–859.

Gesundheit, P., & Macias-Garcia, C. (2005). Biogeografía cladística de la familia Goodeidae (Cyprinodontiformes). In J. Llorente-Bousquets, & J. Morrone (Eds.), *Regionalización biogeográfica en Iberoamérica y tópicos afines* (pp. 319–338). Ciudad de México: Facultad de Ciencias, UNAM.

Gil, D., Graves, J., Hazon, N., & Wells, A. (1999). Male attractiveness and differential testosterone investment in zebra finch eggs. *Science*, *286*(5437), 126–128.

González Zuarth, C., & Macías Garcia, C. (2006). Phenotypic differentiation and pre-mating isolation between allopatric populations of *Girardinichthys multiradiatus*. *Proceedings. Biological sciences/The Royal Society*, *273*(1584), 301–307.

González-Forero, M. (2014). An evolutionary resolution of manipulation conflict. *Evolution*, *68*(7), 2038–2051.

Griggio, M., Morosinotto, C., & Pilastro, A. (2009). Nestlings' carotenoid feather ornament affects parental allocation strategy and reduces maternal survival. *Journal of Evolutionary Biology*, *22*, 2077–2085.

Haldane, J. B. S. (1922). Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics*, *12*(2), 101–109.

Hendrich, B., & Tweedie, S. (2003). The methyl-CpG binding domain and the evolving role of DNA methylation in animals. *Trends in Genetics*, *19*(5), 269–277.

Hollenberg, F., & Wourms, J. P. (1995). Embryonic growth and maternal nutrient sources in goodeid fishes (Teleostei: Cyprinodontiformes). *Journal of Experimental Zoology*, *271*, 379–394.

Juhua, Y., Xuefeng, C., Jianlin, L., Yongkai, T., Hongxia, L., Pao, X., & Zaijie, D. (2010). Isolation of IGF2 and association of IGF2 polymorphism with growth trait in genetically improved farmed tilapias, *Oreochromis niloticus* L. *Aquaculture Research*, *41*(11), e743–e750.

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology and Evolution*, *17*(5), 230–241.

Killian, J. K., Nolan, C. M., Wylie, A. A., Li, T., Vu, T. H., Hoffman, A. R., & Jirtle, R. L. (2001a). Divergent evolution in M6P/IGF2R imprinting from the Jurassic to the Quaternary. *Human Molecular Genetics*, *10*(17), 1721–1728.

Killian, J. K., Nolan, C. M., Stewart, N., Munday, B. L., Andersen, N. A., Nicol, S., & Jirtle, R. L. (2001b). Monotreme IGF2 expression and ancestral origin of genomic imprinting. *Journal of Experimental Zoology*, *291*(2), 205–212.

Kornfeld, S., & Mellman, I. (1989). The biogenesis of lysosomes. *Annual Review of Cell Biology*, *5*, 483–525.

Lau, M. M. H., Stewart, C. E. H., Liu, Z., Bhatt, H., Rotwein, P., & Stewart, C. L. (1994). Loss of the imprinted IGF2/cation-independent mannose 6-phosphate receptor results in fetal overgrowth and perinatal lethality. *Genes and Development*, *8*(24), 2953–2963.

Lawton, B. R., Sevigny, L., Obergfell, C., Reznick, D., O'Neill, R. J., & O'Neill, M. J. (2005). Allelic expression of IGF2 in live-bearing, matrotrophic fishes. *Development Genes and Evolution*, *215*, 207–212.

Lawton, B. R., Carone, B. R., Obergfell, C. J., Ferreri, G. C., Gondolphi, C. M., Vandeberg, J. L., … O'Neill, M. J. (2008). Genomic imprinting of IGF2 in marsupials is methylation dependent. *BMC Genomics*, *9*, 205.

Lim, Y. W., Sanz, L. A., Xu, X., Hartono, S. R., & Chédin, F. (2015). Genome-wide DNA hypomethylation and RNA:DNA hybrid accumulation in Aicardi-Goutières syndrome. *eLife*, *4*, e08007.

Lombardi, J., & Wourms, J. P. (1985a). The trophotaenial placenta of a viviparous goodeid fish. I: Ultrastructure of the Internal Ovarian Epithelium, the Maternal Component. *Journal of Molecular Biology*, *184*, 277–292.

Lombardi, J., & Wourms, J. P. (1985b). The trophotaenial placenta of viviparous goodeid fish. II. Ultrastructure of the trophotaenia, the embryonic component. *Journal of Morphology*, *184*(3), 293–309.

Macías Garcia, C. (2014). Mode of reproduction, mate choice, and species richness in goodeid fish. In R. H. Macedo, & G. Machado (Eds.), *Sexual selection: Perspectives and models from the neotropics* (pp. 253–288). San Diego: Academic Press.

Macías Garcia, C., Smith, G., González Zuarth, C., Graves, J. A., & Ritchie, M. G. (2012). Variation in sexual dimorphism and assortative mating do not predict genetic divergence in the sexually dimorphic Goodeid fish *Girardinichthys multiradiatus*. *Current Zoology*, *58*(3), 440–452.

Macías-Garcia, C., & Saborío, E. (2004). Sperm competition in a viviparous fish. *Environmental Biology of Fishes*, *70*(3), 211–217.

Méndez, E., Planas, J. V., Castillo, J., Navarro, I., & Gutiérrez, J. (2001). Identification of a type II insulin-like growth factor receptor in fish embryos. *Endocrinology*, *142*(3), 1090–1097.

Moore, T., & Haig, D. (1991). Genomic imprinting in mammalian development: A parental tug-of-war. *Trends in Genetics*, *7*(2), 45–49.

Murrell, A., Heeson, S., & Reik, W. (2004). Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. *Nature Genetics*, *36*(8), 889–893.

Nolan, C. M., Mccarthy, K., Eivers, E., Jirtle, R. L., & Byrnes, L. (2006). Mannose 6-phosphate receptors in an ancient vertebrate, zebrafish. *Development Genes and Evolution*, *216*, 144–151.

Oldroyd, B. P., Allsopp, M. H., Roth, K. M., Remnant, E. J., Drewell, R. A., & Beekman, M. (2014). A parent-of-origin effect on honeybee worker ovary size. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1775), 20132388.

O'Neill, M. J., Ingram, R. S., Vrana, P. B., & Tilghman, S. M. (2000). Allelic expression of IGF2 in marsupials and birds. *Development Genes and Evolution*, *210*, 18–20.

O'Neill, M. J., Lawton, B. R., Mateos, M., Carone, D. M., Ferreri, G. C., Hrbek, T., … O'Neill, R. J. (2007). Ancient and continuing Darwinian selection on insulin-like growth factor II in placental fishes. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(30), 12404–12409.

Ortíz-Barrientos, D., Counterman, B. A., & Noor, M. A. F. (2007). Gene expression divergence and the origin of hybrid dysfunctions. *Genetica*, *129*(1), 71–81.

Parker, G. A. (1979). Sexual selection and sexual conflict. In M. Blum & N. Blum (Eds.), *Sexual Selection and Reproductive Competition in Insects* (pp. 123–166). New York, NY: New York Academy Press.

Parker, G. A. (2006). Sexual conflict over mating and fertilization: An overview. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *361*(1466), 235–259.

Reik, W., Constância, M., Fowden, A., Anderson, N., Dean, W., Ferguson-smith, A., … Sibley, C. (2003). Regulation of supply and demand for maternal nutrients in mammals by imprinted genes. *The Journal of Physiology*, *547*(1), 35–44.

Reznick, D. (1981). "Grandfather Effects": The genetics of interpopulation differences in offspring size in the mosquito fish. *Evolution*, *35*(5), 941–953.

Rice, W. R., & Holland, B. (1997). The enemies within: Intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behavioral Ecology and Sociobiology*, *41*, 1–10.

Ritchie, M. G., Hamill, R. M., Graves, J. A., Magurran, a. E., Webb, S. a., & Macías Garcia, C. (2007). Sex and differentiation: Population genetic divergence and sexual dimorphism in Mexican goodeid fish. *Journal of Evolutionary Biology*, *20*, 2048–2055.

Rogers, J. F., & Dawson, W. D. (1970). Foetal and placental size in a Peromyscus species cross. *Journal of Reproduction and Fertility*, *21*(2), 255–262.

Rowe, L., Cameron, E., & Day, T. (2003). Detecting sexually antagonistic coevolution with population crosses. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *270*, 2009–2016.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Alkaline lysis plasmid miniprep protocol*. New York: Cold Spring Harbor Laboratory Press.

Schindler, J. (2005). Digestion of embryotrophic protein in Goodeid teleosts. In M. C. Uribe, & H. J. Grier (Eds.), *Viviparous fishes* (pp. 266–267). Florida: New Life Publication.

Schrader, M., Fuller, R., & Travis, J. (2013). Differences in offspring size predict the direction of isolation asymmetry between populations of a placental fish. *Biology Letters*, *9*, 20130327.

Schrader, M., & Travis, J. (2008). Testing the viviparity-driven-conflict hypothesis: Parent-offspring conflict and the evolution of reproductive isolation in a poeciliid fish. *The American Naturalist*, *172*(6), 806–817.

Schrader, M., & Travis, J. (2009). Do embryos influence maternal investment? Evaluating maternal-fetal coadaptation and the potential for parent-offspring conflict in a placental fish. *Evolution*, *63*(11), 2805–2815.

Schrader, M., Travis, J., & Fuller, R. C. (2011). Do density-driven mating system differences explain reproductive incompatibilities between populations of a placental fish? *Molecular Ecology*, *20*(19), 4140–4151.

Shikano, T., Nakadate, M., & Fujio, Y. (2000). An experimental study on strain combinations in heterosis in salinity tolerance of the guppy *Poecilia reticulata*. *Fisheries Science*, *66*(4), 625–632.

Shull, G. H. (1908). The composition of a field of Maize. *Journal of Heredity*, *4*(1), 296–301.

Stearns, S. (1992). *The evolution of life histories*. New York: Oxford University Press.

Templeton, A. R., Hemmer, H., Mace, G., Seal, U. S., Shields, W. M., & Woodruff, D. S. (1986). Local adaptation, coadaptation, and population boundaries. *Zoo Biology*, *5*, 115–125.

Trexler, J. C., & DeAngelis, D. L. (2003). Resource allocation in offspring provisioning: An evaluation of the conditions favoring the evolution of matrotrophy. *American Naturalist*, *162*(5), 574–585.

Trivers, R. L. (1972). Parental investment and sexual selection. *Selection & the descent of man* (pp. 134–179). Aldine de Gruyter: New York, NY.

Trivers, R. L. (1974). Parent-offspring conflict. *American Zoologist*, *14*(1), 249–264.

Webb, S. A., Graves, J. A., Macias-Garcia, C., Magurran, A. E., Foighil, D. Ó., & Ritchie, M. G. (2004). Molecular phylogeny of the livebearing Goodeidae (Cyprinodontiformes). *Molecular Phylogenetics and Evolution*, *30*(3), 527–544.

Wilkins, J. F., & Haig, D. (2003). What good is genomic imprinting: The function of parent-specific gene expression. *Nature Reviews Genetics*, *4*(5), 359–368.

Wolf, J. B. (2013). Evolution of genomic imprinting as a coordinator of coadapted gene expression. *Proceedings of the National Academy of Sciences*, *110*(13), 5085–5090.

Wolf, J. B., & Brodie, E. D. III (2009). The coadaptation of parental and offspring characters. *Evolution*, *52*(2), 299–308.

Wolf, J. B., Oakey, R. J., & Feil, R. (2014). Imprinted gene expression in hybrids: Perturbed mechanisms and evolutionary implications. *Heredity*, *113*(2), 167–175.

Wourms, J. P., Grove, B., & Lombardi, J. (1988). The maternal-embryonic relationship in viviparous fishes. In W. Hoar, & D. Randall (Eds.), *Fish physiology* (pp. 1–134). New York: Academic Press.

Xie, B., Zhang, L., Zheng, K., & Luo, C. (2009). The evolutionary foundation of genomic imprinting in lower vertebrates. *Chinese Science Bulletin*, *54*(30430370), 1354–1360.

Yuan, X.-N., Jiang, X.-Y., Pu, J.-W., Li, Z.-R., & Zou, S.-M. (2011). Functional conservation and divergence of duplicated insulin-like growth factor 2 genes in grass carp (*Ctenopharyngodon idellus*). *Gene*, *470*(2011), 46–52.

Zeh, D. W., & Zeh, J. A. (2000). Reproductive mode and speciation: The viviparity-driven conflict hypothesis, *22*(10), 938–946.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Saldivar Lemus Y, Vielle-Calzada J-P, Ritchie MG, Macías Garcia C. Asymmetric paternal effect on offspring size linked to parent-of-origin expression of an insulin-like growth factor. *Ecol Evol*. 2017;00:1–10. https://doi.org/10.1002/ece3.3025