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Postnatal expression of *IGF2* is the norm in amniote vertebrates

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The insulin and insulin-like signalling (IIS) network plays an important role in mediating several life-history traits, including growth, reproduction and senescence. Although insulin-like growth factors (IGFs) 1 and 2 are both key hormones in the vertebrate IIS network, research on IGF2 in juveniles and adults has been largely neglected because early biomedical research on rodents found negligible IGF2 postnatal expression. Here, we challenge this assumption and ask to what degree IGF2 is expressed during postnatal life across amniotes by quantifying the relative gene expression of *IGF1* and *IGF2* using publicly available RNAseq data for 82 amniote species and quantitative polymerase chain reaction on liver cDNA at embryonic, juvenile and adult stages for two lizard, bird and mouse species. We found that (i) *IGF2* is expressed postnatally across amniote species and life stages—often at a higher relative expression than *IGF1*, contradicting rodent models; (ii) the lack of rodent postnatal *IGF2* expression is due to phylogenetic placement, not inbreeding or artificial selection; and (iii) adult *IGF2* expression is sex-biased in some species. Our results demonstrate that *IGF2* expression is typical for amniotes throughout life, suggesting that a comprehensive understanding of the mechanisms mediating variation in life-history traits will require studies that measure both IGFs.

1. Introduction

> Mulla had lost his ring in the living room. He searched for it for a while, but since he could not find it, he went out into the yard and began to look there. His wife, who saw what he was doing, asked: 'Mulla, you lost your ring in the room, why are you looking for it in the yard?' Mulla stroked his beard and said: 'The room is too dark and I can't see very well. I came out to the courtyard to look for my ring because there is much more light out here.'
>
> — Farzad, *Classic Tales of Mulla Nasreddin* [1]

The fable above describes a phenomenon defined as the 'street lamp' effect [2], where there is a tendency to search for answers where it is easy to look. Yet, the easiest place to look may not always be the correct place to search. Once a scientific discovery illuminates a hypothetical street lamp, it often defines the focus of the research community and inadvertently discourages researchers from searching outside the 'pool of light'. This effect initiates biases in our research perspective. Here, we illuminate such a bias that has arisen in the study of molecular mechanisms regulating life-history traits and their trade-offs.

The insulin and insulin-like signalling (IIS) network has been well established in laboratory model organisms to regulate the pace of life from embryonic development to ageing [3–5], and the importance of this network is now recognized in the study of natural populations and the evolution of life-history traits [5–7]. This signalling network is activated by the paralogous hormones insulin (INS), insulin-like growth factor (IGF) 1 and IGF2. IGF1 and IGF2 are produced in the liver for endocrine function, while produced in other tissues (e.g. muscle, brain,

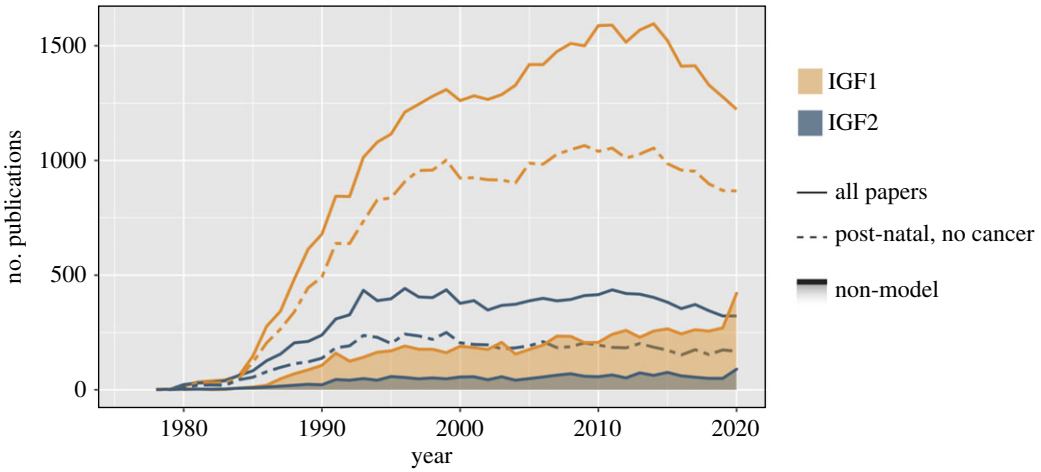

Figure 1. Research bias on the study of IGF1 relative to IGF2. This graph shows the number of publications on either IGF1 or IGF2 each year resulting from a PubMed search using MESH terms. Solid lines represent the total number of IGF1 (light grey in print, orange online) and IGF2 (dark grey in print, blue online) publications that were experimental. Dashed lines represent the proportion of papers at postnatal stages, excluding those performed in cancer research. Shaded curves represent the proportion of papers that were not on biomedical models or humans.

reproductive tissues) for more localized function [8]. The binding of the IGF hormones to cell surface IGF1 receptor (IGF1R) and the insulin receptors (INSR), both insulin receptor A and insulin receptor B isoforms, in target tissues stimulates signalling through the IIS network to promote biological processes such as growth, cellular proliferation, tissue formation and reproduction [8–12]. Decreased signalling through the IIS network is associated with stress resistance and increased longevity [13–16]. In mammals, the IGF2R (a co-opted mannose-6-phosphate receptor) is known as a negative regulator of the IIS because it removes the IGF2 hormone from circulation for degradation [17].

IGFs were first studied in the laboratory rodent models *Mus musculus* and *Rattus norvegicus* [18,19], where it was established that *IGF1* is highly expressed postnatally [20,21], and that *IGF2* is highly expressed during embryonic development but down-regulated to an undetectable level shortly after birth [21–23]. IGF1 has been studied extensively (figure 1; solid lines, unshaded) in the context of postnatal growth, maturation, body size, ageing and as a mediator of life-history trade-offs [12,24–26]. Early research on *IGF2* synthesis has exposed a pattern of paternal imprinting in mammals, elucidating a fascinating relationship for sex-conflict resolution during embryonic development [27], although it should be noted that currently, to our knowledge, there is no evidence of IGF2 imprinting outside of mammals. Due to these findings, the research on *IGF2* has been largely focused on embryonic development [28,29] and the function and evolution of the mammalian placenta [8,30]. Outside of embryonic development, the study of *IGF2* has been largely limited to mis-regulation in the development of cancer [31,32], leaving the roles of *IGF2* in the regulation of postnatal growth, reproduction, senescence and potential trade-offs among these life-history traits under-explored (figure 1, dashed lines).

While the rates of IGF1 and IGF2 publications were relatively equal from their discovery in 1978 to 1984, IGF1 experimentation increased rapidly between 1984 and 2020, far outpacing studies on IGF2. However, because laboratory rodent models lack expression of IGF2 postnatally and there is a lack of molecular resources for study of IGF2 outside of rodent models, publications on IGF2 stagnated. Publication numbers on IGF2 remained consistently lower than IGF1 from the early 1990s through 2020, comprising only 29.9% of all publications on IGFs (figure 1; solid lines, unshaded).

The relative proportions of publications on IGF1 and IGF2 (figure 1) illustrate that studies on laboratory rodent models lit a 'street lamp' defining IGF1 as *the* IGF hormone regulating IIS function postnatally. As *IGF2* is highly expressed postnatally in humans [20], and plasma IGF2 protein is found to be 10-fold higher than IGF1 in adults [33], it is possible that IGF2 may also play an important role in influencing growth and other life-history traits after birth in humans and other vertebrates outside of the laboratory rodents, but research in this area has been largely neglected.

Traditionally, biomedical research forges the path for molecular research in functional and evolutionary ecology, and in this case, this established path has impacted the study of IGF2 not only in humans, but also in physiological ecology and functional genomics. Mirroring the pattern seen in the biomedical field, the proportion of studies focusing on IGF1 remains consistently higher than IGF2 in species outside of rodents and humans (figure 1; shaded areas). Recently, it has been documented that *IGF2* is expressed postnatally in reptile species [6,7,34,35]. In the light of these results, here we ask whether postnatal gene expression of IGF2 across amniote vertebrates is the exception or the norm.

To address this question, we examined postnatal gene expression of *IGF2* in reptiles, birds and mammals, by (i) mining liver transcriptome data to determine relative *IGF1* and *IGF2* gene expression patterns across 82 species from the amniote phylogeny, and (ii) quantifying liver gene expression of both *IGF1* and *IGF2* across life stages in six representative reptile, bird and mammalian species. Our aim is to determine the overall prevalence of *IGF2* postnatal expression across the amniote phylogeny and to further detail *IGF1* and *IGF2* gene expression patterns across embryonic, juvenile and adult stages in other species outside of laboratory rodent models.

2. Methods

All supplemental methods, supplemental tables, data files and code are available in the GitHub repository: https://github.

com/Schwartz-Lab-at-Auburn/IGFs-Across-Amniotes/blob/main/README.md.

(a) Liver RNAseq survey

Amniotic liver ribonucleic acid sequencing (RNAseq) samples were identified using the NCBI Short Read Archive (SRA). For each species, up to four individuals from control conditions were used. When possible, we took two male and two female samples. Raw reads were downloaded, trimmed for quality and mapped to an *IGF1* and *IGF2* reference transcript of the coding sequence from a focal species in the clade (e.g. Carnivora to American black bear, Aves to zebra finch; electronic supplementary material, table S1). Runs that had low numbers of cleaned reads resulting in no mapping to either IGF1 or IGF2 were removed from the study, resulting in a final sample size of 245 SRA runs representing 82 species (electronic supplementary material, table S1). Read counts were normalized by reference transcript size (details in electronic supplementary material, methods). For each individual, we calculated the proportion of IGF1 and IGF2 expression relative to the total IGF expression. These proportions for each gene were averaged across individuals for each species (electronic supplementary material, method details and code in GitHub Repository).

(b) Gene expression across life stages using quantitative polymerase chain reaction

Quantitative gene expression analysis was completed on two birds (zebra finch, *Taeniopygia guttata*; house sparrow, *Passer domesticus*), two lizards (brown anole, *Anolis sagrei*; eastern fence lizard, *Sceloporus undulatus*) and two rodents (house mouse, *M. musculus*; deer mouse, *Peromyscus maniculatus*) across a series of life stages (embryo, juvenile, adult). Our sample sizes ($n = 2$ to 4 within species/age group) are powered for detection and general expression level. Total RNA was isolated from liver samples, quantified and reverse transcribed. Using a relative standard curve and species-specific quantitative polymerase chain reaction (qPCR) primers (electronic supplementary material, table S2), the expression of IGF1 and IGF2 was quantified using qPCR.

To test for differences in relative gene expression of *IGF1* and *IGF2* at each life stage, data were grouped by species and subsequently by life stage (embryo, juvenile, adult) and analysed separately. A linear mixed-effect model [36] was used to analyze the relative differences between copy number of the genes (*IGF1*, *IGF2*). Individual was included as a random effect to account for sample triplicates during qPCR analysis. Sex was included as an independent variable at the adult life stage and as an interaction term when at least two of each sex were available for analysis. When there was a significant interaction between gene and sex, the two sexes were then separated for analysis. Methodological details, statistical code and data are provided in the GitHub Repository.

3. Results

(a) Postnatal liver RNAseq survey

The RNAseq data available in the SRA database provided reasonable coverage of species across the major clades in Mammalia and Reptilia, although some of the smaller clades are only represented by a single species and in some cases a single RNAseq run (electronic supplementary material, table S1; figure 2). Across the 82 amniote species for which we were able to download and map the liver postnatal RNAseq data, we found that only four species did not have detectable (less than 0.01%) postnatal *IGF2* expression; two were laboratory rodents, the house mouse and the brown rat, and the remaining two were the European hedgehog and the Asian house shrew (figure 2). Strikingly consistent across the three inbred and five outbred mouse strains, there was no postnatal expression of *IGF2* detected (figure 3*a*). Interestingly, we found three species, the snapping turtle, Chilean tinamou and Sichuan partridge, that had no detectable *IGF1* expression and an additional eight species from across mammals and reptiles that had very low *IGF1* expression (less than 2% of total *IGF1* expression, figure 2). It is noteworthy that because the reads were being mapped to a reference from another species within the clade, if the nucleotide sequences for *IGF1* or *IGF2* were quickly evolving in that clade (e.g. *IGF1* in squamates [35]), it may decrease the number of reads that were able to map and thus underestimate the expression abundance. Across the amniote phylogeny there is considerable variation in the relative levels of *IGF1* and *IGF2*, but consistently we see *IGF2* expressed postnatally across both mammal and reptile clades, in striking contrast to the pattern seen in the laboratory rodents.

(b) Quantitative reverse transcriptase polymerase chain reaction

The postnatal expression patterns seen in the RNAseq survey were verified in each of our qPCR analyses on two birds, two lizards and two rodents across three life stages (electronic supplementary material, table S2), and we expand on those findings statistically, along with comparisons at the juvenile and embryonic stages.

(i) Birds

Consistent with the RNAseq survey, both the zebra finch and the house sparrow expressed *IGF1* and *IGF2* in adulthood. Within the house sparrow, there was no statistically significant difference in relative expression between the two genes at adulthood ($p = 0.612$) or the juvenile stage ($p = 0.184$) (figure 4*a* and table 1). Adult house sparrow samples were limited to three females and a single male; therefore, no gene by sex comparison was performed. Within the zebra finch, during embryonic development, *IGF2* was expressed at a significantly higher level than *IGF1* ($p < 0.0001$). By contrast, during the juvenile life stage, *IGF1* was the predominantly expressed gene ($p = 0.005$). While there was no statistically significant difference in relative expression at the adult stage based on an alpha value of 0.05, both gene ($p = 0.056$) and a gene by sex interaction ($p = 0.083$) neared significance. Due to marginal significance with our limited sample size and the moderate effect size, we analysed each sex separately and found that *IGF2* was expressed at a significantly higher level than *IGF1* in male finches ($p = 0.014$) but not female finches (figure 4*a* and table 1).

(ii) Lizards

The Eastern fence lizard exhibited significantly higher expression of *IGF1* during the juvenile life stage ($p = 0.002$). By adulthood (male samples only), there was no statistically detectable difference in relative *IGF1* and *IGF2* expression ($p = 0.962$) (figure 4*b* and table 1). In comparison, in the brown anole lizard, there was no statistical difference in relative expression of *IGF1* and *IGF2* at either the embryonic ($p = 0.286$)

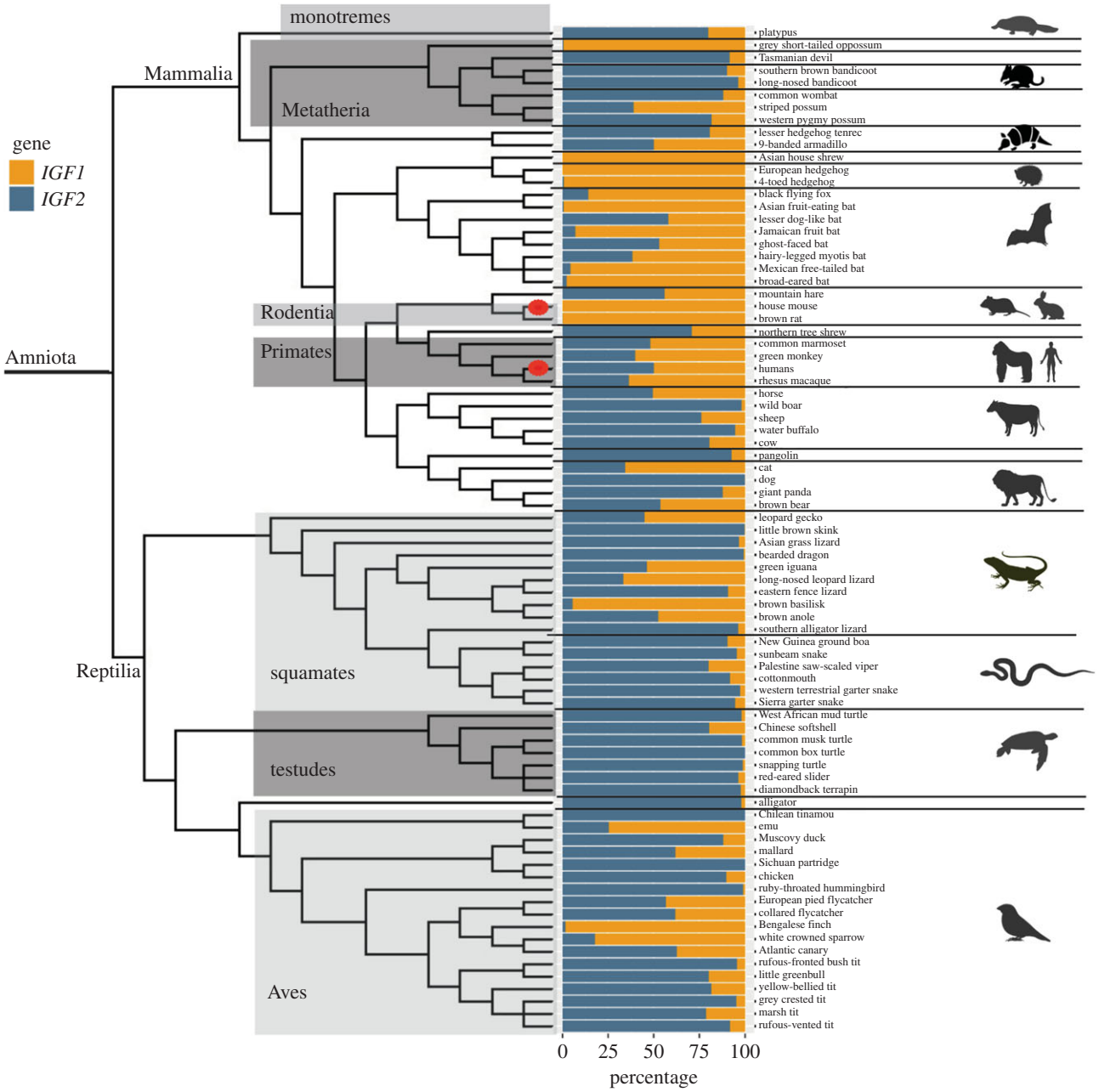

Figure 2. Relative IGF1 and IGF2 expression across amniotes. The phylogenetic tree represents relationships among the amniote species used in this RNAseq analysis. The branch lengths do not represent evolutionary distance. Orders of interest are labelled and outlined with grey shaded boxes. Red dots indicate house mouse and human values. The horizontal orange/blue bars represent the relative proportions of *IGF1* (orange/light grey in print) and *IGF2* (blue/dark grey in print) gene expression for each species, averaged across all the individuals ($n = 1$–4) of that species. (Online version in colour.)

or the juvenile life stages ($p = 0.233$). By adulthood, *IGF2* expression was significantly higher than *IGF1* ($p = 0.003$). However, there was an interesting gene by sex interaction ($p = 0.010$). It was found that *IGF2* was expressed at a significantly higher level than *IGF1* in females ($p < 0.001$), while there was no statistically detectable difference within males ($p = 0.667$) (figure 4*b* and table 1).

(iii) Rodents

Due to the patterns of IGF expression within adult inbred and outbred mouse strains observed in the RNAseq analysis, we chose to expand our qPCR analysis to both inbred and outbred rodents across life stages. To do so, we used samples from inbred house mouse C57BL/6 J strain embryonic laboratory rodents, inbred house mouse C57BL6 adult laboratory rodents, outbred deer mouse juveniles and outbred deer mouse adults. Similar to what has previously been reported in laboratory rodents, we found a lack of *IGF2* postnatal expression ($p < 0.001$, relative to *IGF1*); *IGF2* was also expressed at a significantly higher level than *IGF1* during embryonic development in the inbred laboratory reared individuals ($p < 0.001$). Interestingly, these patterns persisted in the outbred deer mouse, with *IGF2* expression being nearly undetectable at both the juvenile ($p < 0.001$) and adult ($p < 0.001$) time points (figure 3*b* and table 1).

4. Discussion

Both IGF1 and IGF2 bind the IGF1R and INSR to activate the IIS network [9]. As such, both hormones are critical for mediating variation in life-history traits such as postnatal growth, reproduction and senescence; yet predominately,

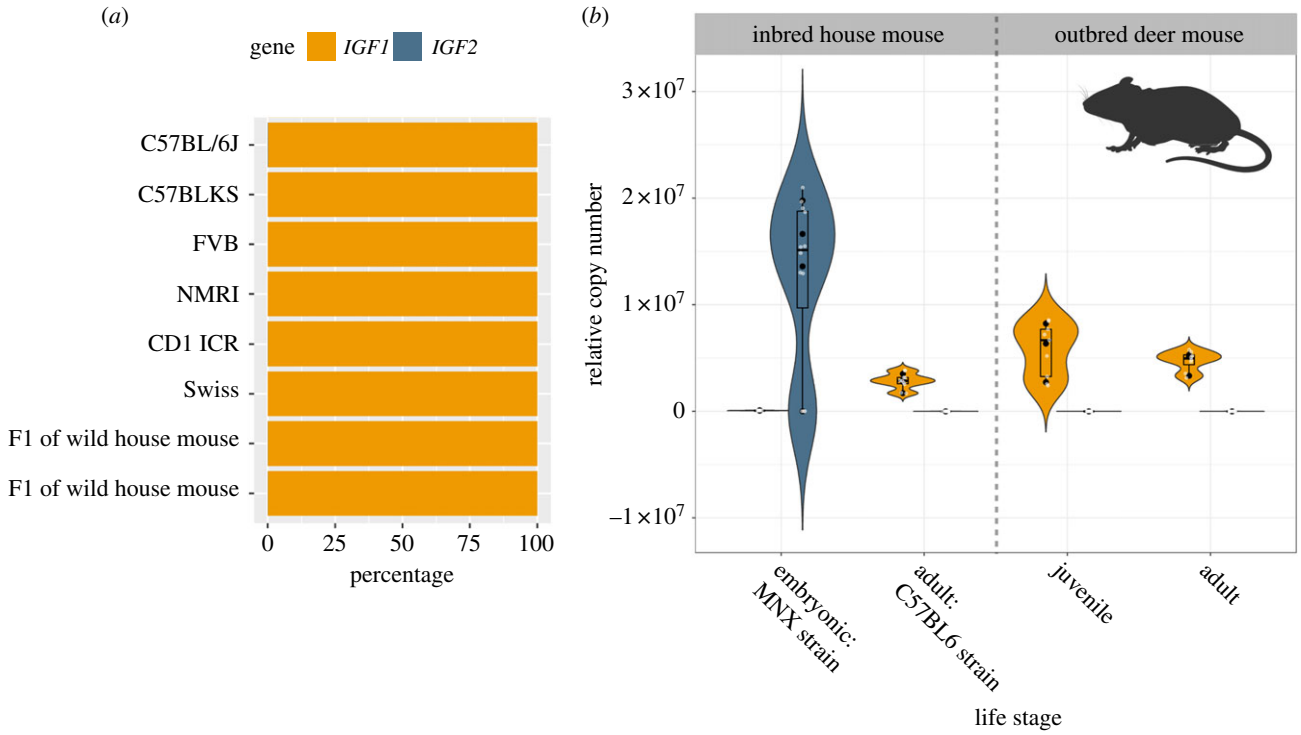

Figure 3. Relative *IGF1* and *IGF2* in inbred and outbred mice. (*a*) Using publicly available RNAseq data, relative levels of *IGF1* (orange) and *IGF2* (blue) expression were calculated for all accessible strains of the laboratory house mouse. Inbred strains are represented in bold text, while outbred strains are shown in unformatted text. (*b*) Quantitative PCR analysis was performed on embryonic and adult laboratory inbred house mouse liver as well as juvenile and adult outbred deer mouse samples. *IGF1* (orange) and *IGF2* (blue) expression is depicted in relative copy number. Triplicate qPCR runs are represented by individual white datapoints, while averages for individuals are represented by a single black datapoint. (Online version in colour.)

the focus has been on IGF1. Here, we clearly demonstrate that *IGF2* is expressed during postnatal development in reptiles, birds and mammals. We also provide evidence that in some species IGF1 and IGF2 age-related expression patterns are sex biased. Taken together, these results suggest that examining variation in IGF2 alongside variation in IGF1 will be crucial for understanding the physiological mechanisms that mediate variation in growth and other life-history traits in vertebrates.

The transcriptomic analysis on 82 species clearly demonstrates that *IGF2* postnatal expression is the 'norm' across the amniote phylogeny, as it was detected in 95% of these species. In fact, most species (56 of the 82 total species) expressed *IGF2* at a level of 50% or greater of total IGF expression. These data confirm that the lack of *IGF2* expression after birth seen in rodents, and perhaps a few other mammalian species, is an exception in the context of the amniote phylogeny. When examining the expression of the *IGF* hormones across the lifespan via quantitative gene expression analysis, we again confirmed that *IGF2* was expressed in bird and lizard species at all life stages—from embryonic development to adulthood—and often at an equal or higher level than *IGF1*.

While existing work examining *IGF2* gene expression and IGF2 protein levels in circulation is limited, our findings are consistent with previous studies performed in both Aves and Squamata. For example, in the wild turkey (*Meleagris gallopavo*), hepatic *IGF2* expression decreased significantly at the time of hatching relative to embryonic expression, but by three weeks post hatching, the expression levels had risen to levels statistically similar to those of late embryonic development [37]. Similarly, studies in brown anoles found *IGF2* is expressed at the embryonic, juvenile and adult stages [34], with males expressing both *IGF1* and *IGF2* at a higher level than females in adulthood [7]. Further, a survey of 18 squamate juvenile liver transcriptomes (also included in this study) found *IGF2* to be expressed in every species [35]. The results presented herein extend and further support these previous findings.

Despite limited sample size and high levels of individual variation, using two different methods, the data clearly demonstrate that *IGF1* and *IGF2* are both expressed across the lifespan in the majority of species examined (all clades), and *IGF2* is often expressed at a higher level than *IGF1* postnatally. Our transcriptomic survey and qPCR results show distinct patterns in the *IGF1* : *IGF2* expression ratios among clades. It is worth noting that our sample sizes within a group are small and should be used to generate hypotheses for future in-depth experiments rather than generalizable conclusions beyond the nearly ubiquitous postnatal expression of *IGF2* across non-rodent species. We would like to highlight a few hypotheses that may be fruitful for future investigation due to the coincidence of unique traits that are linked to IIS regulation and interesting IGF1 : IGF2 patterns in particular clades. Intriguingly, our results illuminate two clades (bats and turtles) that are known to be outliers in longevity (which can be regulated by IIS signalling), with each clade having a strong bias in the relative proportion of IGF1 : IGF2 postnatal expression (bat clade bias towards *IGF1*, turtle clade bias towards *IGF2*) [38,39]. Another example is snakes, a carnivorous sub-group within squamates that is variable in diet types. Snakes had a strong bias towards *IGF2* expression, which raises the question of whether IIS metabolic signalling and hormone-receptor evolutionary relationships may be influenced by

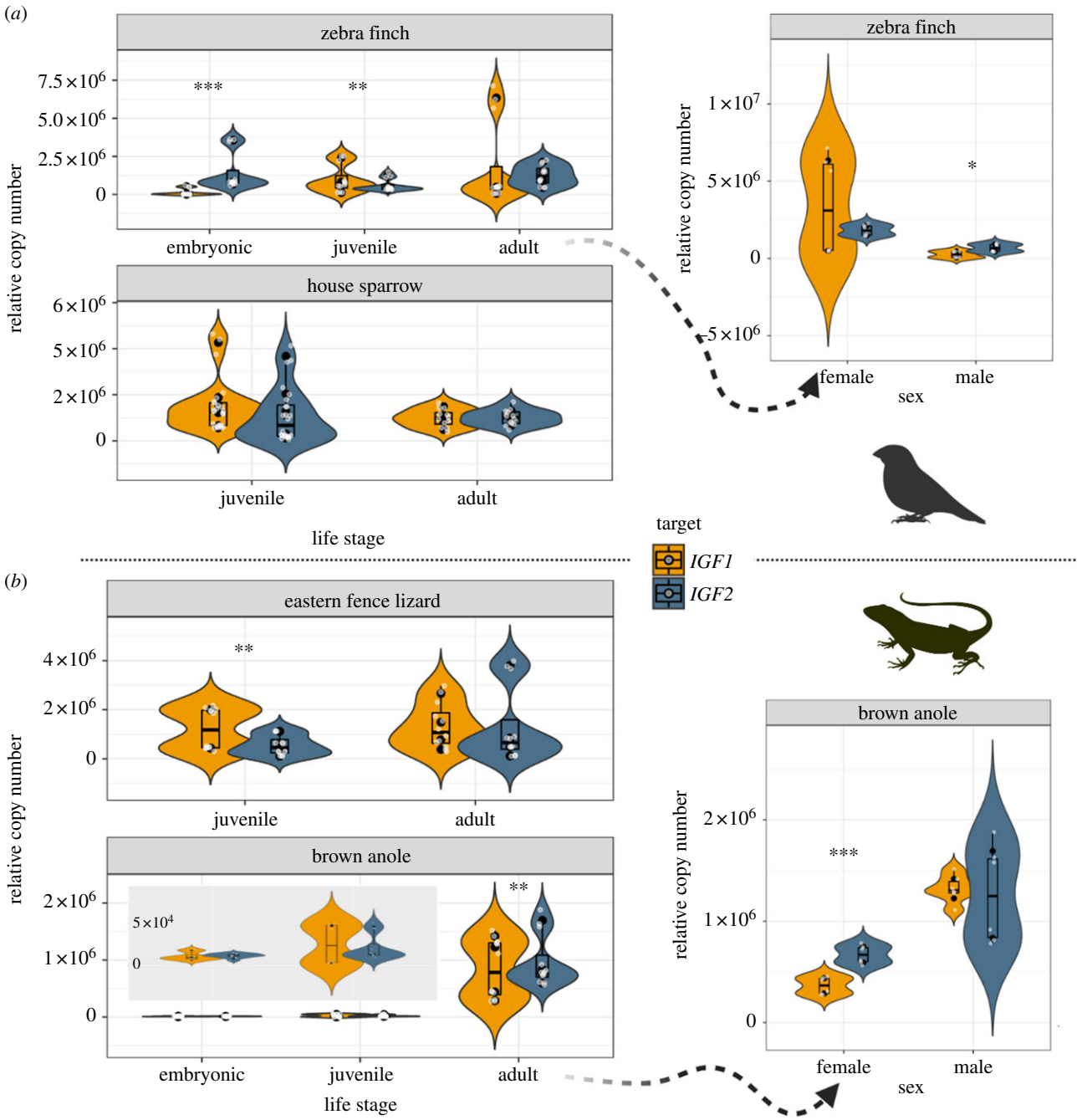

Figure 4. Relative expression of *IGF1* and *IGF2* across life stages. (*a*) Aves. Quantitative PCR analysis was completed on liver samples from the zebra finch at three life stages and the house sparrow at two life stages. (*b*) Squamates. Quantitative PCR analysis was completed on liver samples from the brown anole at three life stages and the eastern fence lizard at two life stages. To see the relative expression levels more clearly in the brown anole plot, an inset of the embryonic and juvenile life stages is shown in grey. For all plots, *IGF1* (orange/light grey in print) and *IGF2* (blue/dark grey in print) expression is depicted in relative copy number. Triplicate qPCR runs are represented by individual white data points, while averages for individuals are represented by a single black data point. When there was a significant interaction between sex and gene expression, the sexes were plotted separately. Significance is indicated with an asterisk (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). (Online version in colour.)

diet type. Phylogenetically controlled experiments would be necessary to test these relationships and whether they are biologically relevant.

Interestingly, our qPCR results also suggest there is sex-biased expression in adult zebra finches and brown anoles. In the zebra finch, only adult males expressed *IGF2* at a higher level than *IGF1*, whereas in brown anoles, only adult females displayed higher *IGF2*. Additionally, in female zebra finches *IGF1* varied greatly in expression levels (but not statistically different in mean expression to *IGF2*), while male *IGF2* expression was highly variable in the brown anole. Sex-biased expression and within sex variation in expression of these hormones potentiate them having unique roles in regulating sex-specific differences in growth, reproductive status and senescence. While these findings are intriguing and may be biologically significant, with the limited sample size in this study, the relationships should be explored further in future studies.

Rodent models have led to significant advances in our understanding of the IIS network; however, our results elucidate the distinct limitations in the applicability of rodent models in IIS research. If *IGF2* were expressed in outbred

Table 1. Statistical results from qPCR analysis. Comparisons evaluate relative expression of *IGF1* and *IGF2* at each life stage and between sexes when appropriate. Dashed bars in sex-specific comparison column indicate no statistical test was performed. P-values of statistical significance ($p < 0.05$) are highlighted in bold text.

species	age	sex-specific comparison	estimate ± s.d.	favoured expression	*p*-value
zebra finch	embryonic	—	1 391 307 ± 249 475	IGF2	**<0.0001**
	juvenile	—	−379 543.5 ± 119 870.6	IGF1	**0.005**
	adult	—	−1 602 247.0 ± 783 851.0	IGF1	0.056
		gene × sex interaction	2 025 798.0 ± 1 108 533.0	IGF2	0.084
		male	423 551.0 ± 138 612.4	IGF2	**0.014**
		female	−1 602 247.0 ± 1 098 905.0	IGF1	0.179
house sparrow	juvenile	—	−346 245.4 ± 255 461.0	IGF1	0.184
	adult	—	50 766.3 ± 97 480.5	IGF2	0.612
eastern fence lizard	juvenile	—	−760 675.0 ± 210 606.5	IGF1	**0.002**
	adult	—	−9152.3 ± 189 742.0	IGF1	0.962
brown anole	embryonic	—	1047.3 ± 952.6	IGF2	0.286
	juvenile	—	−5569.1 ± 4501.1	IGF1	0.233
	adult	—	308 578.4 ± 90 239.0	IGF2	**0.003**
		gene × sex interaction	−365 100.8 ± 127 617.1	IGF1	**0.010**
		male	−56 522.5 ± 127 065.4	IGF1	0.667
		female	308 578.4 ± 11 854.4	IGF2	**<0.001**
mouse	embryonic (house mouse)	—	16 312 472.0 ± 847 373.0	IGF2	**<0.001**
	juvenile (deer mouse)	—	−5 648 981.0 ± 606 787.2	IGF1	**<0.001**
	adult (house and deer mouse)	—	−3 684 151.0 ± 328 946.5	IGF1	**<0.001**

house mouse or deer mouse, this would allow for the use of the extensive resources for the laboratory mouse such as existing knockout strains, antibodies, and quantification methods to study IGF2 in other rodents. However, as we dug deeper into mouse expression patterns, we found that none of the nine rodent samples with publicly available RNAseq data, nor the postnatal samples collected from inbred laboratory house mice or outbred deer mice examined through quantitative PCR displayed detectable *IGF2* expression. While the lack of mouse and rat postnatal *IGF2* expression is similar to what has been shown previously [21,40], our results demonstrate persistence of this rodent pattern regardless of inbreeding status or evolutionary clade across rodent families (Muridae and Cricetidae). These results further illustrate that when it comes to understanding the functional effect of the IIS network and the interactions between the *IGF* hormones, rodent models are the exception to the norm of postnatal *IGF2* expression and thereby a poor model to address these questions.

The results presented here demonstrate the street lamp effect that has occurred in the study of the hormone regulators of the IIS network. The scientific community at large has known about the postnatal co-expression of *IGF1* and *IGF2* in humans [20,41–44] and other species [35,45] (figure 1). Yet, the limitations of biomedical rodent models have focused our attention on IGF1 hindering our understanding of IGF2's functional and physiological impact. Importantly, as both IGF1 and IGF2 hormones can bind, and compete for binding, to the IGF1R and INSR to regulate signalling to promote growth and reproduction, by only studying one of these hormones we are getting an incomplete picture. As a field, our understanding of how these paralogous hormones complete within the organism is highly biased by mammalian-based investigations. It is assumed that IGF1, IGF2 and INS bind and complete for access to the cellular receptors universally; yet, outside of mammals, namely rodents, there is very little information on how these hormone–receptor relationships vary across clades. These assumptions, as well as the evolution of such relationships across clades, have yet to be examined.

Further, these hormones are pleiotropic, and their functions may change with sex, age, energetic status, stress and reproductive state, requiring controlled experiments to elucidate their individual functions. Experiments manipulating levels of IGFs through processes such as supplemental injections, gene editing through clustered regularly interspaced short palindromic repeat (CRISPR) and cell culture experimentation can be used to understand functions and consequences on phenotypes, physiology and fitness, as well as how those relationships evolve across species groups. To properly conduct these experiments, large technical advances are also necessary. Assays that can reliably detect IGF1 and IGF2 independently in order to accurately measure these hormones with minimal cross-talk are essential in studying their relationships in response to biotic and abiotic variables. But, the first step is awareness that the bias is present; it is then up to researchers to look beyond the current pool of light. We hope the results presented here, showing near ubiquitous postnatal expression of IGF2

across the amniote clade, encourage the biological community to start talking about, and studying, IGF2 and its role in mediating variation in life-history strategies.

Data accessibility. RNAseq data are available on NCBI. All supplemental methods, supplemental tables, data files and code are available in the GitHub repository: https://github.com/Schwartz-Lab-at-Auburn/IGFs-Across-Amniotes/blob/main/README.md.

The data are also provided in electronic supplementary material [46].

Authors' contributions. A.B.: data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; A.M.R.: data curation, formal analysis, investigation, writing—review and editing; H.W.: resources, writing—review and editing; B.H.: resources, writing—review and editing; W.R.H.: resources, writing—review and editing; T.S.S.: conceptualization, data curation, formal analysis, funding acquisition, investigation, project administration, resources, supervision, validation, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

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