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读书报告

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LETTER

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Insulin resistance in cavefish as an adaptation to a nutrient-limited environment

Misty R. Riddle[1]*, Ariel C. Aspiras[1]*, Karin Gaudenz[2], Robert Peuß[2], Jenny Y. Sung[2], Brian Martineau[1], Megan Peavey[1], Andrew C. Box[2], Julius A. Tabin[1], Suzanne McGaugh[3], Richard Borowsky[4], Clifford J. Tabin[1] & Nicolas Rohner[2,5]

洞穴鱼的胰岛素抵抗是对营养限制环境的一种适应

IF=41.577

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Introduction

Introduction

- **Periodic food shortages are a major challenge faced by organisms in natural habitats.**

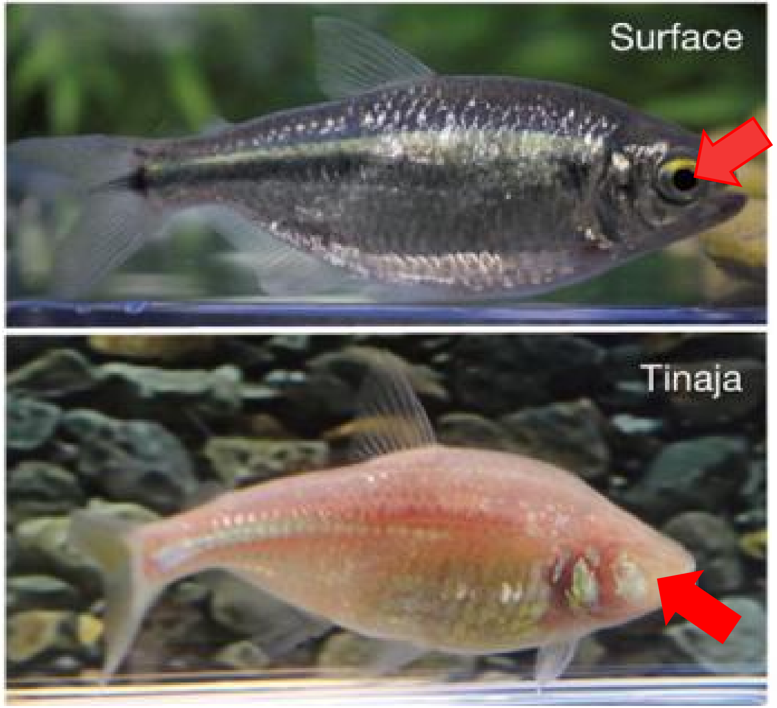
  周期性的食物短缺是自然栖息地生物面临的一个主要挑战。

- **Cave-dwelling animals must withstand long periods of nutrient deprivation, as—in the absence of photosynthesis—caves depend on external energy sources such as seasonal floods.**

  穴居动物必须经受长时间的营养短缺，因为在没有光合作用的情况下，只能依赖于外部能源，如季节性洪水。

Introduction

地表鱼



穴居鱼

Figure 1 a, Surface fish and Tinaja cavefish of *A. mexicanus*.

The fish species *A. mexicanus* consists of interfertile river-dwelling and cave-dwelling populations (Fig. 1a)， here referred to as 'surfacefish' and 'cavefish', that experience markedly different nutrient availablility .

墨西哥丽脂鲤可分为河居（地表水体）种群和穴居种群。文章中称作"地表鱼"和"洞穴鱼"它们的营养利用率有明显不同。

Cavefish are resistant to starvation.

洞穴鱼对饥饿有抵抗力。

Introduction

- **when fooddeprived, cavefish lose a smaller fraction of their body weight compared to surface fish.**

  当食物剥夺时，洞穴鱼类的体重比地表鱼类减少的少。

- **Several factors have been identified that contribute to starvation resistance, including reduced metabolic circadian rhythm, decreased metabolic rate and elevated body fat.**

  已有研究报道了几个有助于饥饿抵抗的因素，包括代谢昼夜节律减少，代谢率降低和体脂增加。

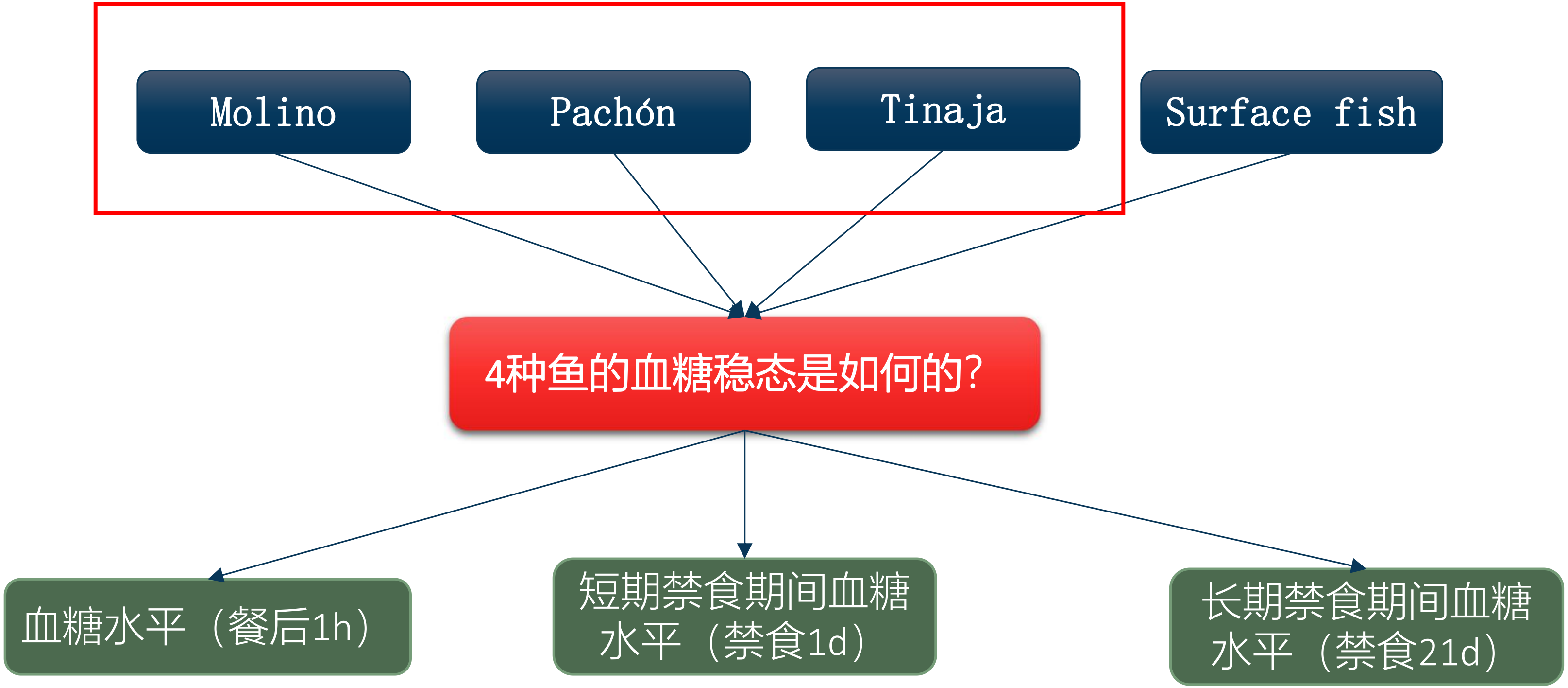
Introduction

血糖调节是代谢调节的一个关键调节，而本研究聚焦于Tinaja, Pachón and Molino这3种洞穴鱼(3种鱼都以它们的栖息地命名)，它们都来源于两种地表鱼入侵洞穴进化而来，其中Tinaja, Pachón比 Molino 更为古老。并发现洞穴鱼有血糖稳态失调和胰岛素抵抗。

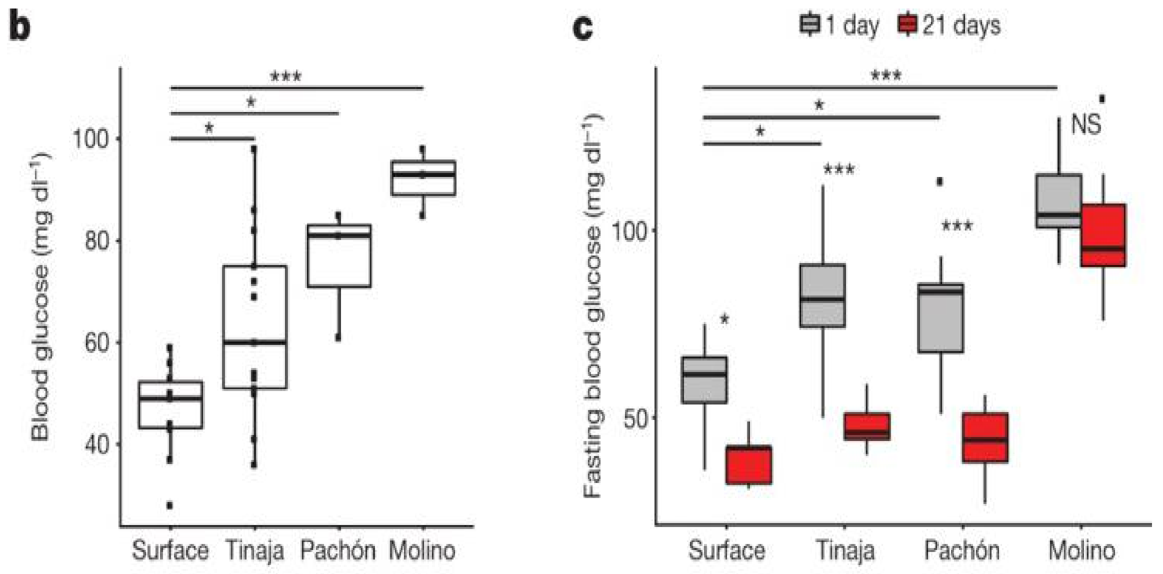
02

Research Design and Result

Research Design and Result



Research Design and Result



b, **Blood glucose** (1 h postprandial) in surface fish compared to cavefish (*n*=10,13, 3 and 3, respectively, for surface fish, Tinaja, Pachón and Molino cavefish).

c, **Fasting blood glucose at day 1 versus day 21** (*n*=20 per population and condition).

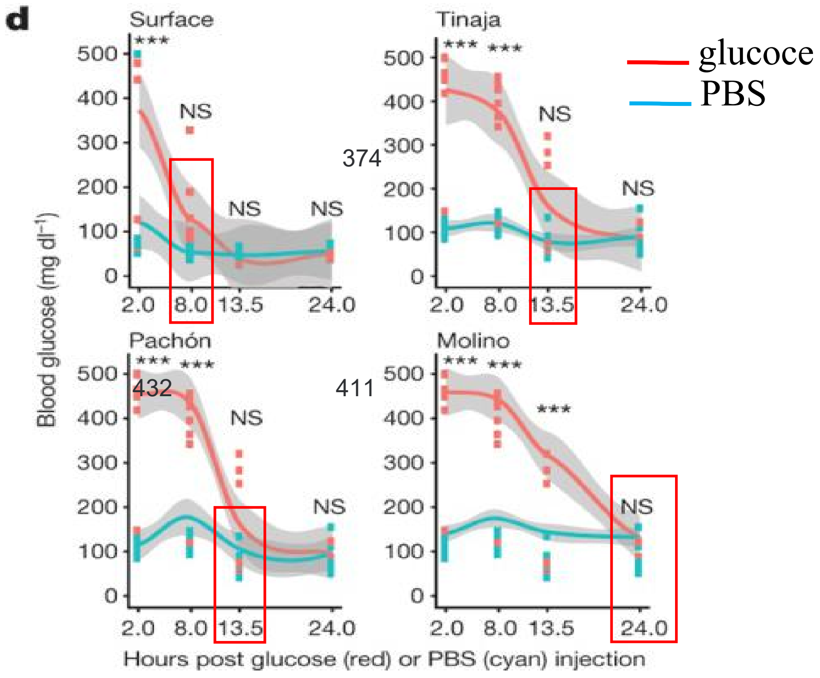
禁食21d后**Tinaja, Pachón**的血糖水平明显下降；**surface fish** 只显示轻微的下降；**Molino cavefish**维持高血糖水平，突出了M种群洞穴鱼的代谢适应与我们调查的其他两种洞穴鱼种群之间的根本区别。

results suggest that dysregulated glucose homeostasis is a common feature of cavefish populations.
研究结果表明，葡萄糖稳态失调是穴居鱼类种群的一个共同特征。

Research Design and Result

为了验证葡萄糖稳态失调是穴居鱼类种群的共同特征这一假设

葡萄糖耐量：腹腔注射葡萄糖，（2.5 mg/gram fish），来比较葡萄糖稳态的急性调控。



d, Glucose tolerance test. Blood glucose after intraperitoneal injection of glucose (red) or PBS (blue). Data points represent values for individual fish and grey shade indicates 95% confidence interval for polynomial regression.

Our results suggest that cavefish have impaired glucose clearance.

洞穴鱼的葡萄糖清除能力受损。

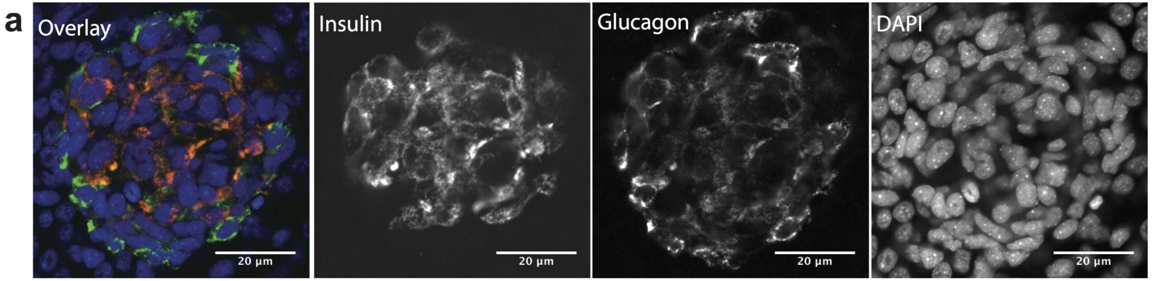
Research Design and Result

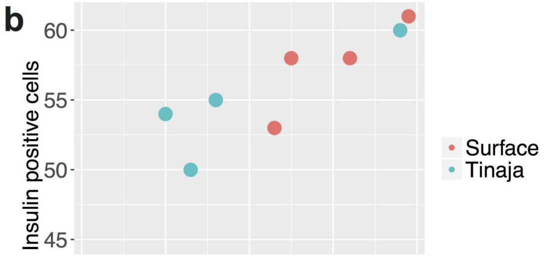
Glucose homeostasis requires the balanced release of **insulin** and **glucagon** that instructs tissues to absorb glucose from the blood or produce glucose from stored glycogen.

葡萄糖稳态需要胰岛素和胰高血糖素的平衡释放，它指示组织从血液中吸收葡萄糖或从储存的糖原中产生葡萄糖。

- **胰岛素和胰高血糖素定位和定量。**
- 注射Arginine（精氨酸）（可以刺激胰高血糖素和胰岛素同时释放。）
- **注射重组人胰岛素。**

Research Design and Result





c

	Surface	Tinaja	p–value
glucagon cells	54	50	0.678
insulin cells	54	52	0.275
fish length (mm)	6.8	6.5	0.780
insulin/glucagon ratio	1.0	1.1	0.636

a, Whole-mount immunohistochemical detection of insulin- and glucagon-positive cells in Tinaja larvae at 10days post fertilization.

b, Number of glucagon- and insulin-positive cells in surface and Tinaja larvae at 10–11 days post fertilization (*n*=5 fish per population,).

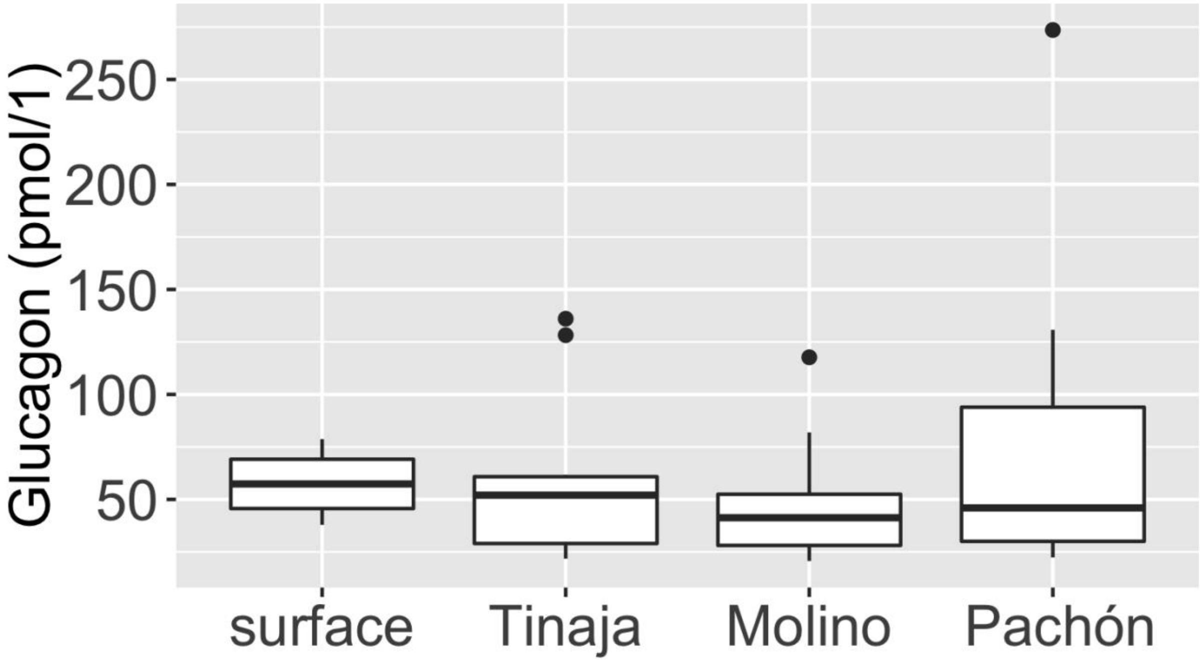
c, Average number of glucagon- and insulin-positive cells, fish length, ratio of insulin to glucagon positive cells and *P* value comparing the surface and Tinaja values (determined using Student's *t*-test).

Extended Data Figure 1 | Numbers of insulin- and glucagon-positive cells in the developing pancreas are unchanged in Tinaja cavefish relative to surface fish.

在发育中的胰腺中胰岛素和胰高血糖素阳性细胞的数量与地表鱼相比没有变化。

这说明地表鱼类和穴居鱼类的胰腺发育相似。释放胰岛素和胰高血糖素的细胞数量并未受到影响。

Research Design and Result

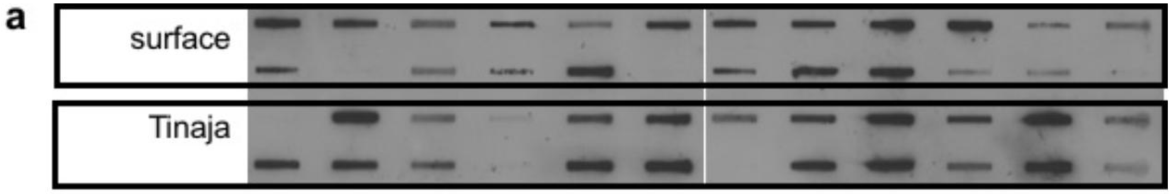


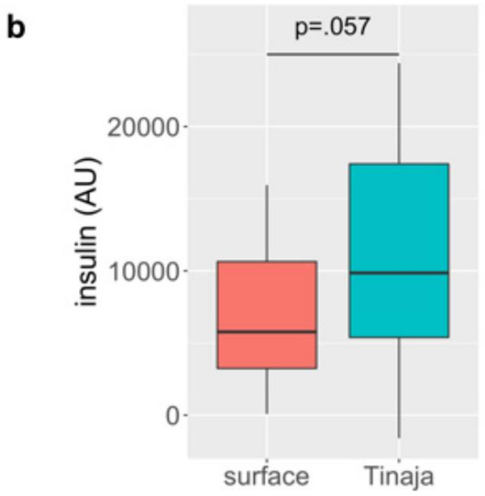
Extended Data Figure 2 | Serum glucagon levels are comparable between the different populations.

4种鱼的血清胰高血糖素水平没有显著性变化。

Box plot comparing serum glucagon levels between surface, Tinaja, Molino and Pachón fish after 24-h fast. *n*=12 fish per population, average of 57.87, 59.76, 79.66 and 48.89 respectively. *P*=0.52, one-way ANOVA.

Research Design and Result





Extended Data Figure 3 | Serum insulin levels are comparable between surface and Tinaja fish.

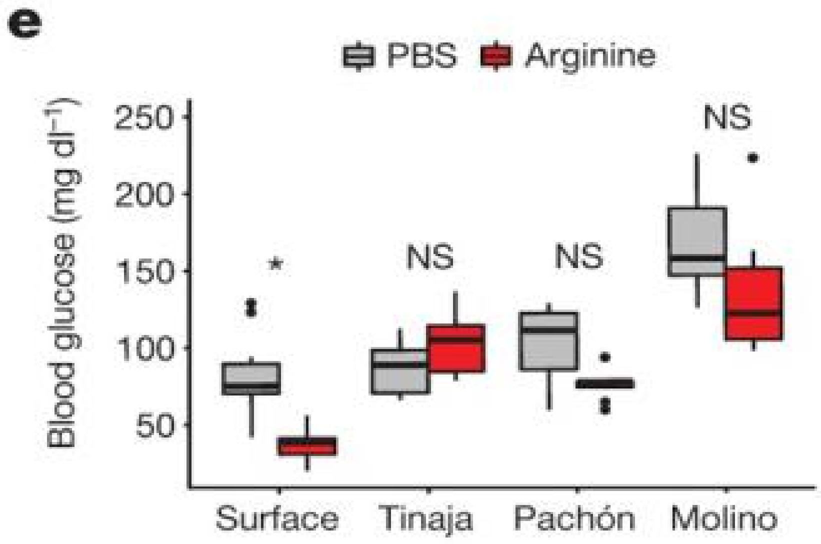
a, Serum blotted onto nitrocellulose membrane using Bio-Dot SF microfiltration apparatus probed with anti-insulin antibody (DAKO). Each blot represents an individual fish between 1- and 2-years-old (*n*=24 fish per population).

b, Quantification of insulin level measured by densitometry of blots.

Tinaja洞穴鱼和地表鱼的血清胰岛素水平没有显著差异。

Research Design and Result

精氨酸腹腔注射实验



e, Blood glucose 5 h after intraperitoneal injection of arginine (*n*=10 per population and condition).

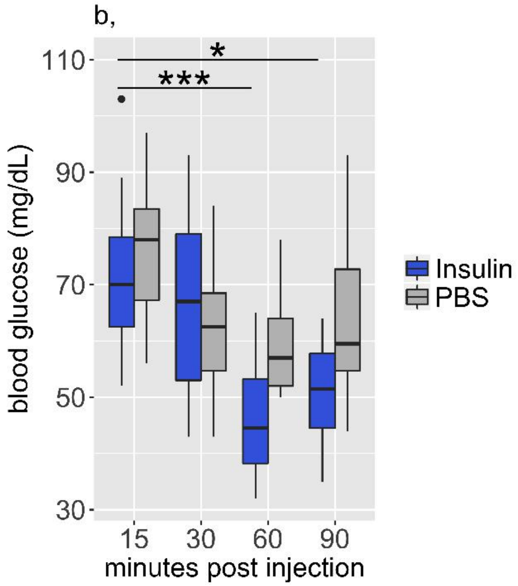
精氨酸注射（6.6 uM/gram fish）5h后血糖情况。

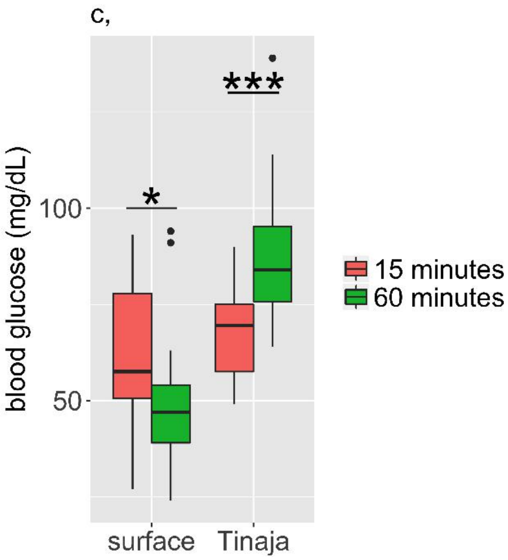
Arginine（精氨酸）：可以刺激胰高血糖素和胰岛素同时释放。

结果显示，精氨酸注射后，虽然地表鱼血糖水平显著降低但洞穴鱼血糖水平并没有改变。

Research Design and Result

注射重组人胰岛素 （0.06g/mg fish）





Extended Data Figure 4 | Insulin decreases blood glucose level in surface fish.

b, Blood glucose levels of surface fish over time, after injection of PBS or insulin.

c, Blood glucose levels at 15 and 60 min after insulin injection in surface fish and Tinaja cavefish.

注射重组人胰岛素60min后，地表鱼的血糖明显下降，而洞穴鱼的血糖没有下降。

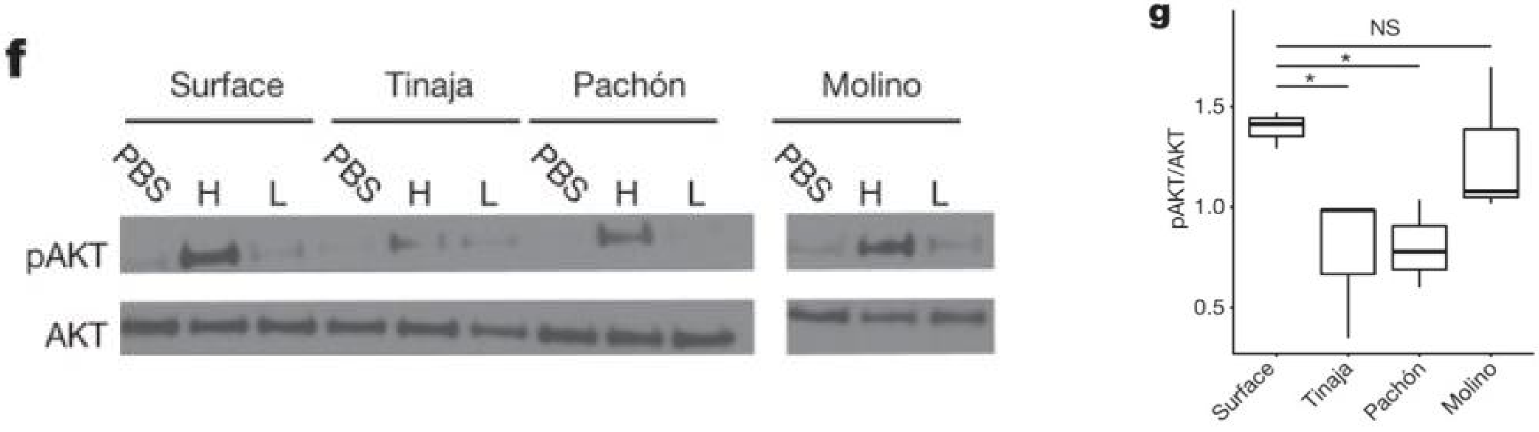
小结：

Our combined observations that glucagon and insulin levels do not differ between surface and cavefish, and that cavefish do not decrease blood glucose levels in response to arginine or insulin, suggest that cavefish may be insulin resistant.

地表鱼和洞穴鱼之间的胰高血糖素和胰岛素水平没有差异，而精氨酸或胰岛素刺激后洞穴鱼血糖水平并没有明显降低，这表明洞穴鱼可能存在胰岛素抵抗。

Research Design and Result

胰岛素刺激葡萄糖摄取通过AKT (pAKT)信号通路进行。



f, Western blot: cell lysates probed with pAKT (ser473) and AKT antibodies. Lysates produced from skeletal muscle treated *ex vivo* with PBS, a high (H, 9.5–11.5μg ml$_{-1}$) or a low (L, 0.95–1.15μg ml$_{-1}$)level of insulin.

g, Quantification of bands by densitometry of highest concentration treatment (*n*=3 per population).

我们的结果表明，这表明与地表鱼相比， Pachon和Tinaja洞穴鱼确实具有胰岛素抵抗能力。 Molino鱼和地表鱼的磷酸化水平相当，但是，Tinaja和Pachon鱼可能同时进化出血糖调节和胰岛素抵抗的改变，而Molino鱼可能通过不同的机制进化出葡萄糖代谢的改变。

Research Design and Result

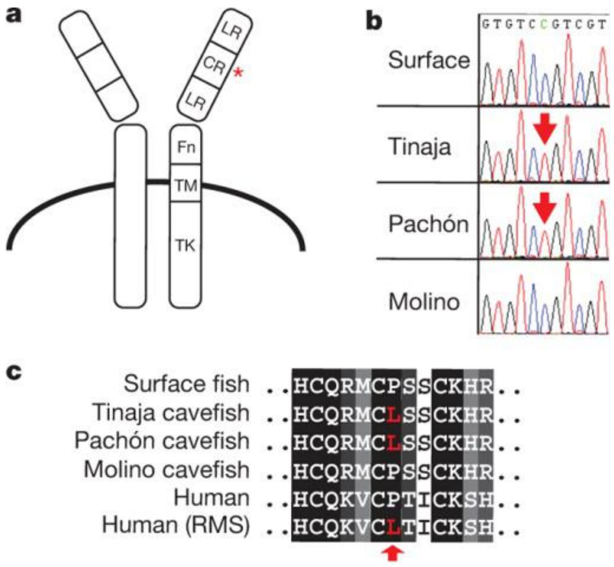
综合上述的“胰岛素和胰高血糖素定位和定量”；“Arginine（精氨酸）注射”；“重组人胰岛素注射”“insulin刺激后，测定AKT（PAKT）磷酸化水平”实验结果，地表鱼和洞穴鱼之间的胰高血糖素和胰岛素水平没有差异。在洞穴鱼中精氨酸或胰岛素刺激不会降低血糖水平，以及洞穴鱼的AKT(PAKT)磷酸化途径受损，这表明洞穴鱼确实存在胰岛素抵抗。

Research Design and Result

洞穴鱼胰岛素抵抗的遗传机制？

从基因组中得到insulin pathway 有关的所有已知的序列。

发现地表鱼和洞穴鱼的insra在半胱氨酸富集区出现点突变，保守脯氨酸突变为亮氨酸(P211L)。



a, Schematic of the insulin receptor .Red asterisk depicts position of the P211L mutation.

b, Sequence chromatogram of the mutation in *Astyanax*.

c, Amino acid alignment of the insulin receptor P211L mutation with patients with Rabson–Mendelhall syndrome (‘Human (RMS)’).

insra富含半胱氨酸区域的点突变是否会降低胰岛素的结合效率?

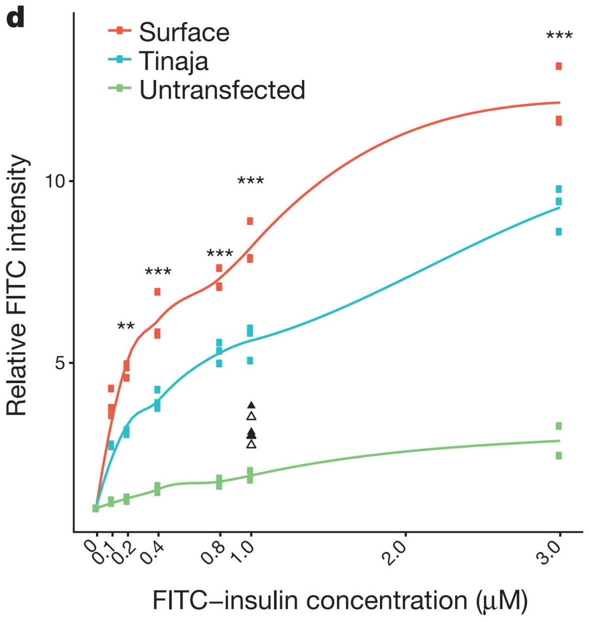
Research Design and Result

如何证明？

转基因HEK293T (Flp-In-293)细胞系（稳定表达地表鱼或洞穴鱼的全长insra）。

荧光素异硫氰酸酯(FITC)标记的人胰岛素孵育细胞。

荧光成像观测荧光强度，即ins的结合效率。

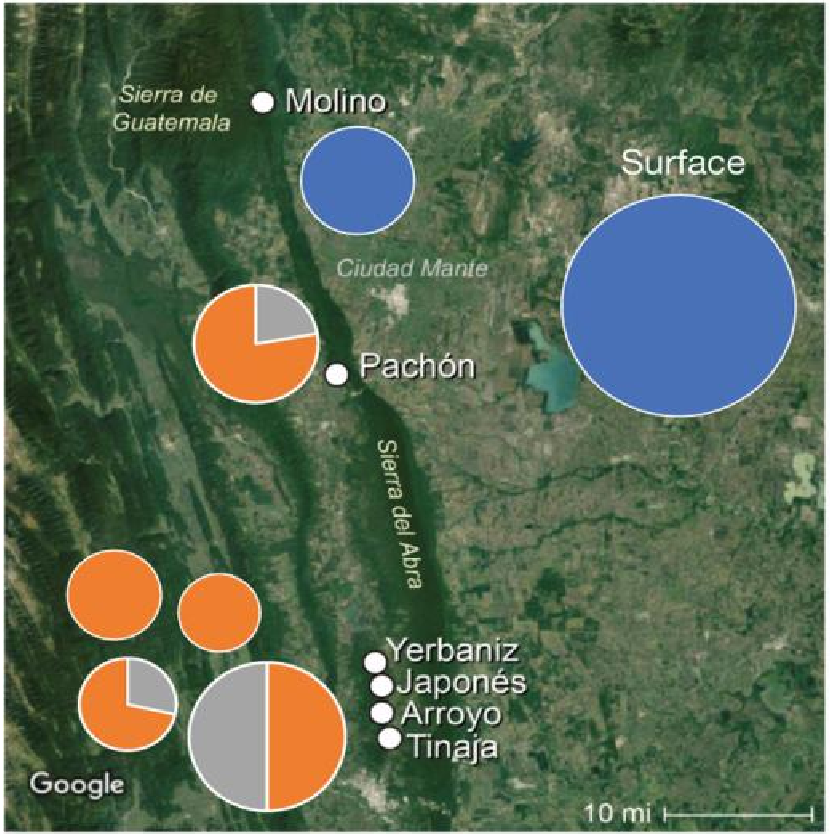


Insra 的 P211L 突变通过改变胰岛素结合效率影响胰岛素信号传导。

d, Relative FITC intensity of cells stably transfected with Flag-tagged surface-fish or Tinaja-cavefish insulin receptor and incubated with FITC-labelled insulin.

Research Design and

接下来测试了野生鱼类中P211L突变的存在和频率。



对来自不同地区的71条地表鱼和来自6个不同地区的51条洞穴鱼进行了基因分型。

与我们上面的结果一致，这种突变在Molino鱼中不存在(n=8)，但在所有其他被测试的洞穴鱼种群中都存在(Tinaja、Yerbaniz、Pachon、Japones和Arroyo，n=36)

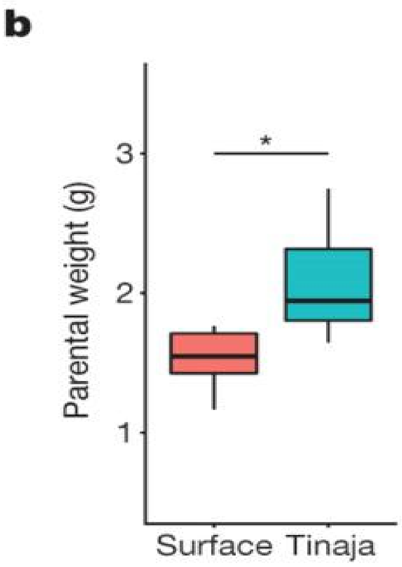
而携带这种突变的洞穴鱼种群全部都来自同一祖先的表面鱼。

a, Map of the region, overlain with genotyping results of wild-caught samples. Pie charts indicate percentage of fish homozygous for surface allele (blue), cave allele (orange) or heterozygous (grey).

Research Design and

We found that cavefish weigh more than surface fish on a nutrient-limited

我们发现在营养限制的环境中，洞穴鱼比地表鱼更重。



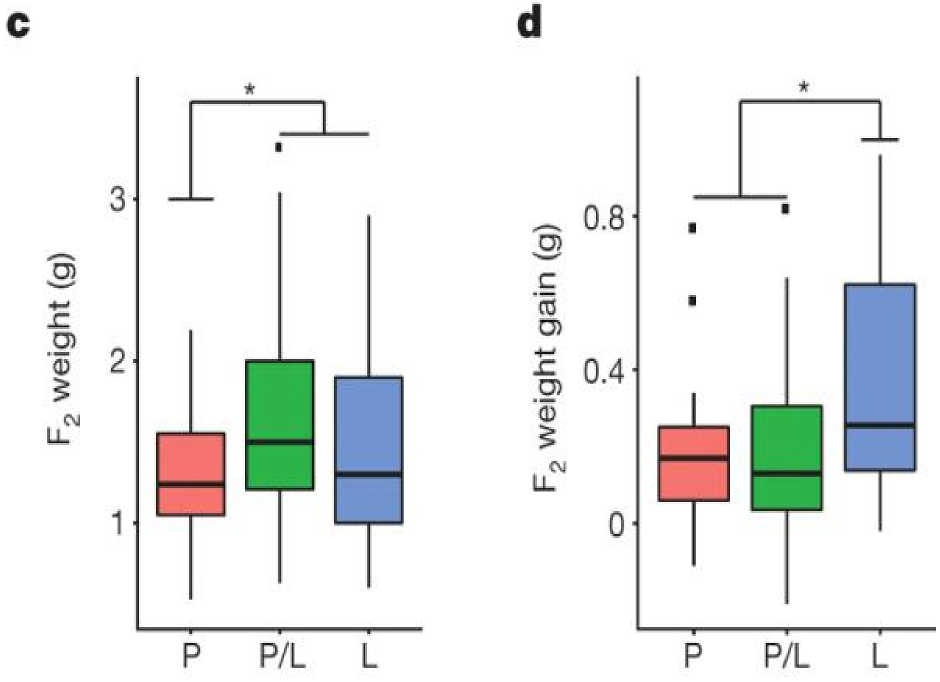
b, Weight of Tinaja males (*n* = 6) and surface males (*n* = 5) on a nutrient-limited diet.

为了证明insra突变是否会影响体重

对124 条surface–Tinaja F2 雄性鱼（约1.5 龄）进行基因分型和测量体重。

为了排除食欲对结果的影响，选择初始体重约相同(<2克）F2鱼，确保每天喂食6毫克的食物，为期4个月。然后测量体重。

Research Design and Result



c, Weight of 18-month old F2 male Tinaja–surface hybrids genotyped for the P211L mutation.

d, Change in weight of F2 Tinaja–surface hybrid males on fixed diet. *n* = 21 (P), 39 (P/L) and 20 (L).

P : P-homozygous surface fish, *n* = 22;

L: L-homozygous cavefish, *n* = 27;

P/L : heterozygotes

洞穴鱼的体重增加可能与insra的突变有关。

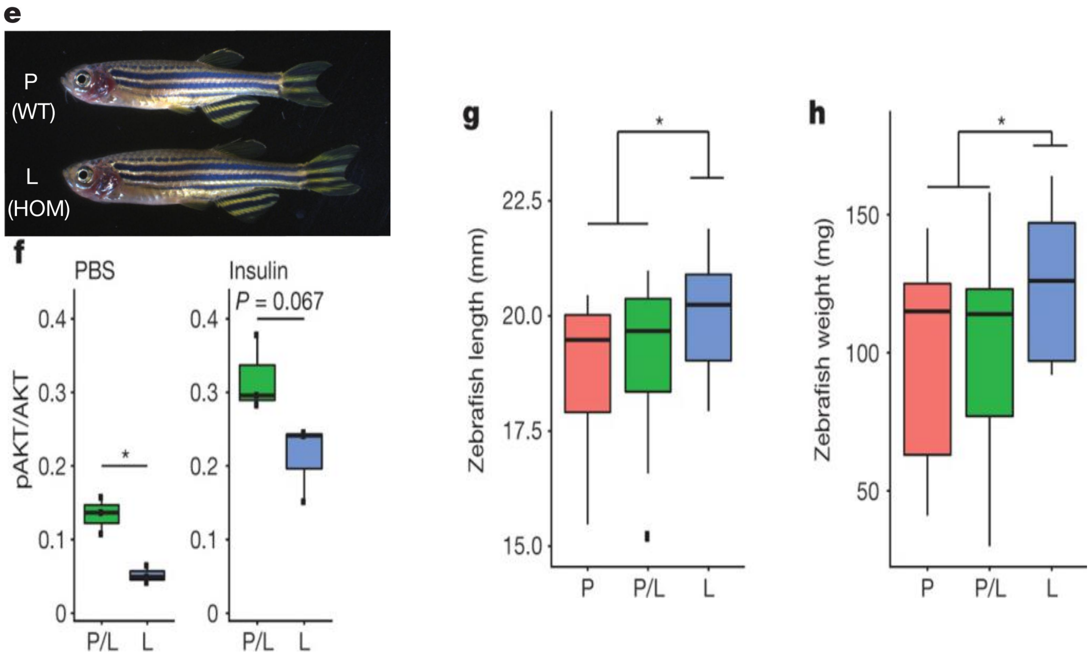
Research Design and Result

如何证实？

洞穴鱼体重增加和胰岛素抵抗是否**确实**是由于Insra突变引起的呢?

利用**CRISPR**基因编辑，通过同源定向修复将该突变基因导入斑马鱼**(Danio rerio)**体内。

对F2斑马鱼进行基因分型，测量insulin/PBS刺激后PAKT/AKT的比值，和3种基因型斑马鱼的体重和体长。



f, Ratio of pAKT:AKT in adult zebrafish skeletal muscle treated *ex vivo* with PBS or insulin (*n* = 3 per genotype and condition).

g, **h**, Length and weight of wild-type zebrafish (*n* = 13 (P)) and heterozygous (*n* = 22 (P/L)) and homozygous (*n* = 11 (L)) P211L mutant zebrafish.

Research Design and Result

上述结果说明了什么问题：

- **Our findings show that the P211L mutation contributes to both the increased weight and insulin resistance observed in Tinaja and Pachón cavefish.**

在**Tinaja and Pachón** 两种类群的洞穴鱼中， **P211L**突变导致了体重增加和胰岛素抵抗。

- **in mammals, full loss-of-function mutations in the insulin receptor are associated with retarded growth and lower levels of body fat.**

在哺乳动物中，使胰岛素受体丧失功能的突变会引起机体生长迟缓和体内脂肪水平降低。

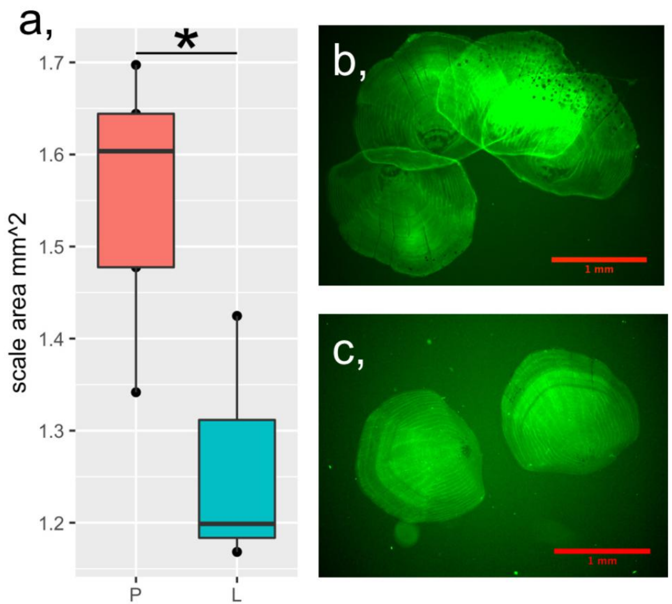
- **diminished insulin signalling has an opposite effect in fish, but the mechanisms leading to the difference remain unclear.**

在鱼类中，胰岛素的敏感性减弱会出现与哺乳动物相反的反应，但导致这种差异的机制还不清楚。

Research Design and Result

洞穴鱼有胰岛素抵抗和高血糖症

- 在人类中，这些表型定义为2型糖尿病。
- 据报道，洞穴鱼有脂肪肝，这也与2型糖尿病有关。
- 本研究也有类似的研究结果——携带insra突变的转基因斑马鱼鳞片尺寸减小。（利用CRISPR基因编辑，通过同源定向修复将该突变导入斑马鱼(Danio rerio)体内。）



这可能暗示了一种进化上的权衡，即牺牲生理健康以获得抗饥饿的能力。

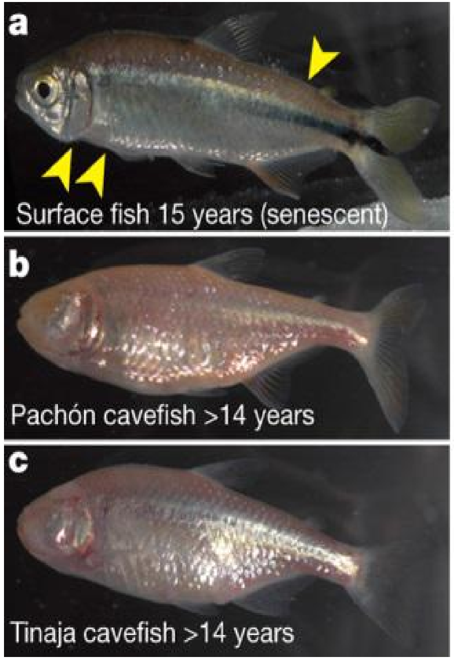
Extended Data Figure 8 | Scale growth is impaired in the insra zebrafish mutant.

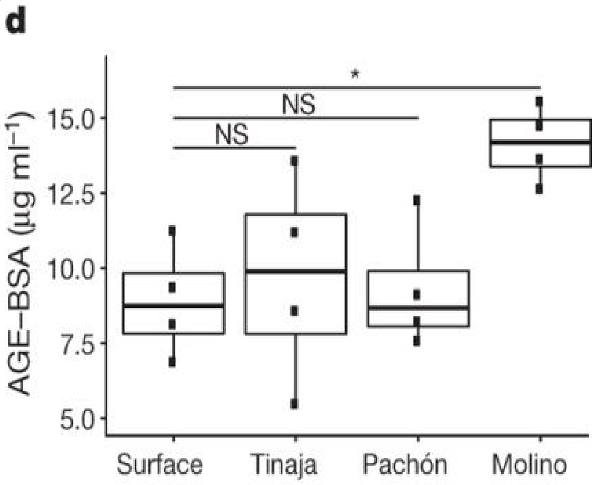
a, Quantification of scale size in zebrafish of the indicated genotype.

b, c, Representative images of scales stained with 0.005% calcein with contrast and brightness adjusted to show scale edges.

Research Design and Result

皮肤凹陷，鱼鳍残破，背部驼背





(**a**),Suface fish

(**b**) Pachón

(**c**) Tinaja

d, Quantification of advanced glycation end-products in serum (AGE–BSA) from approximately two-year-old fish after a three-day fast (*n*=4 for each population).

导致糖尿病患者发病的一个主要原因是血液中大量的非酶促糖化蛋白。它们产生的晚期糖化终末产物(AGEs) 可造成组织损伤。

Despite elevated blood glucose levels and insulin resistance, Tinaja and Pachón cavefish do not show signs of senescence and do not accumulate advanced glycation end-products in the blood. a–c

03

conclusion

conclusion

- **Our findings establish cavefish as a model with which to investigate resistance to pathologies of diabetes-like dysregulation of glucose homeostasis.**

  建立了穴居鱼类作为一种模型，用来研究糖尿病样糖稳态失调的病理抵抗。

- **Moreover, our results highlight the extreme physiological measures that can evolve in critical metabolic pathways to accommodate exceptional environmental challenges**

  结果突出了极端的生理措施，可以在关键的代谢途径演变，以适应特殊的环境挑战。

- **Molino population may have evolved altered blood glucose homeostasis through a different mechanism than did Tinaja and Pachón cavefish.**

  Molino种群可能进化出与Tinaja和Pachon洞穴鱼不同的机制，来调节葡萄糖稳态。

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敬请大家批评指正！

汇报时间：2018年12月09日

汇报人：赵文丽

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