

Ponssa and Abdala provide important new information about a new model for understanding the role of mechanical movement in joint formation. This is an interesting topic that provides data supporting previous literature on the role of biomechanics in joint morphogenesis. It would have been nice to see a similar approach to other animal models to support the current model, but it is unnecessary for this manuscript. Overall, the experimental design is solid and conclusions sound. Is there any way to discuss the frequency of deformation in each experimental group? This should be feasible considering the groups included an N = 5.

I would like a bit more discussion on how this model differs from other models of how movement affects joint morphology. Specifically, how does the movement of the limb relate to the contraction of muscle during limb development. The model used here would still generate resistance on the joint in contrast to other immobility models where muscle contraction is inhibited. Is it the physical bending of a joint that promotes proper morphogenesis?

The writing also became more and more of an issue further into the manuscript. The suggestions below have tried to fix some of the issues, but I suggest the authors try and work on the sentence structure to help the clarity of the writing.

Overall, the figure legends would be much easier to follow if they had letters indicating each figure, if they were oriented in the same direction in every image, and the same (or as close as you can) anatomical structures were highlighted in all of the figures. As it is now, it takes a lot for the reader to try and compare the regions of the joint between images when the orientation is different and different structures are labeled in each image.

It would be very helpful for the reader to have a schematic of the experimental design up front. Possibly in figure 1 a figure similar to the image below would be helpful:

	St. 36	St. 38	St. 40	St. 42	Juvenile
A					
B					
C					
D					
E					

There are several sentences that could be re-written to make the conclusions more clear. Below are suggestions for helping with the clarity:

Page 4, line 61: Please rephrase sentence for clarity

Page 4, line 74: Please rephrase sentence. It is clear, but the sentence is structured in an awkward manner

Page 5, line 85: Too many spaces – “In order to evaluate the role of”

Page 5, line 89: Please rephrase or make it more descriptive – “which were located achievable.”

It is confusing to me how the animals housed in agar can move to eat any food. Did they eat as much food as the controls?

Page 5, line 89: Remove comma – “Both, control and experimental tadpoles feed normally.”

Page 6, line 106: This should be placed in Results rather than methods: “Of the 1 min recorded, the selected experimental tadpoles moved  $5.63 \pm 8.539$  sec (N = 10); meanwhile, controls moved for  $33.2 \pm 18.06$  sec (N = 10) (F = 4.48;  $p < 0.05$ ).”

Page 12, line 240: Remove “the”. I am also unsure what “hip joint raise” means: “Nowlan et al. (2014a, 2015) showed that the shape morphogenesis is advanced prior to cavitation of the hip joint raise”

Page 12, line 244: Remove the “s” in phenotypes: “cavitation and phenotypes modifications”

Page 12, line 255: I don’t think induce is the correct term here. Something like “promotes” or “supports” is more appropriate. Also add the word “to” before the word “induce”: “and this movement would be induce the normal knee-joint formation”

Page 13, line 263: This is a key sentence, but lacks clarity due to grammatical error. Possibly change “originated” to “caused by”. Also “is” to “are”: Thus, we propose that the severe latent problem originated by the lack of movement since the early stages is dramatically manifested from Stages 40–42

Page 13, line 267: Maybe change “and the insensitive to mechanical effects of the early stages” to “and the insensitivity of the early stages to mechanical disruption”

Page 13, line 267: change the word “until” “after”: even when they were transferred to water until the froglet stage”

Page 13, line 284: change “Similar effect was” to “Similar effects were”

Page 14, line 287: I don't think you meant to say the permanent deformity caused immobilization. It should be the other way around: "The authors highlight the significance that brief period of immobilization caused a permanent deformity."

Page 14, line 289: "It is surprising that the response to this particular stimulus (reduced mobility) is independent of the ontogenetic environment of the individuals". I'm not sure if I agree with this statement for the current study as the changes occurred in exact correlation with metamorphosis. Entire animal is changing at this time point, which could have an impact on why stage 40-42 was such a critical window. I think this point should be discussed.

Page 15, line 320: Insert "a" after "also": "allow us to suggest that frogs could be also considered suitable animal model system".

Page 15, line 331: change "of" to "for". Change "some no considered feature" to some feature not considered": "responsible of the alterations of knee-joint development instead of some no considered feature."

Page 22, line 449: Stage 37-38; Rephrase the term "in the histological sample muscle tissue still not completely differentiated is observed"

Page 22, line 454: Remove the comma: "articulation area, where the long-bone articular surfaces begin differentiation"

Page 22, line 455: Incomplete sentence. Add the word "which after comma to correct: "Joint formation is evidenced by the interzone, a close package of mesenchymal cells."

Page 22, line 459: Please label these areas in the figure similar to stage 42: "In the cartilage of the long bones the resting, proliferating and hypertrophic zone can be distinguished."

Page 22, line 161-169: It is difficult to identify which images the authors are referring to without lettering of each image in figure 3. Doing so would really help the clarity of the article.

Page 23, line 470: Please indicate in the figure legend whether the right images are close-up images of independent samples or close-ups of images on the left. They look like new individuals

Page 23: line 473: Is there any way to also label g: graciella sesamoid in figure 1 to assist in comparing the figures.

Page 23: line 478: Please label the images with letters to direct the reader to the correct image: "Juvenile specimen, note the severely damaged lateral articular cartilage in the detail indicated by the curved arrow."