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Sex reversal induces size and performance differences among females of the African pygmy mouse, *Mus minutoides*

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CORRECTION

Correction: Sex reversal induces size and performance differences among females of the African pygmy mouse, *Mus minutoides* (doi: 10.1242/jeb.157552)

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There was an error published in *J. Exp. Biol.* (2017) **220**, 1947-1951 (doi: 10.1242/jeb.157552).

The *in vivo* bite force values were overestimated as a result of incorrect settings of the piezoelectric force transducer used to measure bite forces; all bite force values are 2.4 times higher than they should be. This bias does not affect the statistical results and conclusions of the paper.

Correct versions of Fig. 2 and Table 2 are given below.

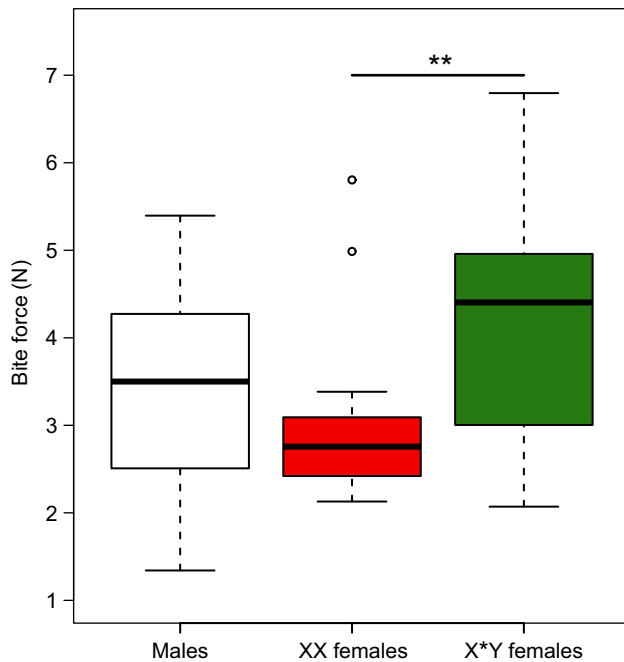


Fig. 2. Boxplot of bite forces of the three *M. minutoides* groups. Data are shown for males ($n=19$), XX females ($n=18$) and X*Y females ($n=17$). Limits of the boxes represent the upper and lower quartiles, the bar is the median, the whiskers represent the maximum and minimum value, or 1.5 times the interquartile distance in cases where outliers are present, and the circles are outliers. Asterisks indicate significant differences between means. Tukey's HSD test revealed a significant increase in bite force in X*Y females compared with XX females ($P<0.01$).

Table 2. Results of Tukey's HSD test for differences in bite force, mandible centroid size and skull centroid size

	Difference	Lower	Upper	P
Bite force				
♂ vs XX ♀	-0.36	-1.27	0.56	0.62
X*Y ♀ vs ♂	0.80	-0.13	1.73	0.1
X*Y ♀ vs XX ♀	1.16	0.22	2.10	0.01
Mandible centroid size				
♂ vs XX ♀	3.15	-20.86	27.16	0.95
X*Y ♀ vs ♂	31.7	7.33	56.07	0.01
X*Y ♀ vs XX ♀	28.55	3.86	53.24	0.02
Skull centroid size				
♂ vs XX ♀	-4.53	-41.21	32.14	0.95
X*Y ♀ vs ♂	67.56	30.33	104.78	<0.01
X*Y ♀ vs XX ♀	72.09	34.38	109.80	<0.01

The authors apologise for any inconvenience this may have caused.

SHORT COMMUNICATION

Sex reversal induces size and performance differences among females of the African pygmy mouse, *Mus minutoides*

Samuel Ginot*, Julien Claude, Julie Perez and Frederic Veyrunes

ABSTRACT

Differences in biological performance, at both intra- and inter-specific levels, have often been linked to morphology but seldom to behavioural or genotypic effects. We tested performance at the intraspecific level by measuring bite force in the African pygmy mouse, *Mus minutoides*. This species displays an unusual sex determination system, with sex-reversed, X*Y females carrying a feminizing X* chromosome. X*Y females cannot be differentiated from XX females based on external or gonadal morphology; however, they are known to be more aggressive. We found that bite force was higher in X*Y females than in other females and males. We then performed geometric morphometric analyses on their skulls and mandibles and found that the higher performance of X*Y females was mainly explained by a greater overall skull size. The effects of the X* chromosome thus go beyond feminization, and extend to whole-organism performance and morphology. Our results also suggest limited effects of behaviour on bite force.

KEY WORDS: Behaviour, Bite force, Geometric morphometrics, Intra-sex competition, Sex reversal

INTRODUCTION

Bite force is a frequently used performance trait in functional morphological studies. Most of these studies have clearly demonstrated the link between osteology, muscular activity and biting performance at the intraspecific as well as interspecific levels (e.g. Herrel et al., 1999; Aguirre et al., 2002; Davis et al., 2010; Santana et al., 2010). Morphology has also been shown to be a driver of performance and fitness via intra-sexual competition (Herrel et al., 2010; Lailvaux et al., 2004). However, to our knowledge, the interactions between genetics, behaviour, morphology and performance at the intraspecific and even intra-sex levels have not yet been studied in mammals.

Here, we investigated bite force and skull morphology in the African pygmy mouse, *Mus (Nannomys) minutoides* Smith 1834. This species is known to have a unusual sex determination system, with three types of sex chromosomes: X, Y and a feminizing X*, producing XY males and XX, XX* females and a high proportion of sex-reversed, X*Y females (Veyrunes et al., 2010). The three types of females present no differences in external morphology or gonad anatomy (Rahmoun et al., 2014), but are known to vary in other traits: X*Y females have a higher reproductive success (Saunders et al., 2014), and have a much more aggressive behaviour

(latency to attack, number of attacks; Saunders et al., 2016) than the XX and XX* females. Because biting is one of the characteristics of attacks that relates to aggressiveness, we compared the bite force of the X*Y females with that of other females and males to assess the effect of their genotypic changes on performance. In parallel, we performed measurements of morphological attributes of the skull and mandible in all genotypes. By doing so, we tried to link chromosomal differences to performance, through potential morphological and/or behavioural (aggressiveness) variations. Examining these interactions in *M. minutoides* could also help improve our understanding of the ecological and evolutionary implications of the emergence of this unusual sex determination system.

MATERIALS AND METHODS

Specimens

All mice were raised in the facilities of Montpellier University (France). The colony was established from wild-caught animals from South Africa (see details in Saunders et al., 2014). Overall, 54 mice were used in the experiments with 19 males and 35 females, all adults. Seventeen females were found to carry a Y chromosome using PCR amplification of the *Sry* gene (following Veyrunes et al., 2010). The 18 others did not have the *Sry* gene, and thus were either XX or XX*. XX and XX* females do not differ in terms of behaviour and reproductive success (Saunders et al., 2014, 2016) and were pooled in this study after checking that they did not represent distinct phenotypic groups. Recognizing all XX* females requires karyotyping, which is technically demanding; instead, we were able to identify eight of them in our dataset thanks to their genealogy. These eight individuals were compared with the other non-X*Y females, and their morphology and bite force were roughly equivalent in mean and variance (results not shown). These XX and XX* females are hereby referred to as 'XX females'. The experimental protocol was performed in accordance with European guidelines and with the approval of the Ethical Committee on Animal Care and Use of France (no. CEEA-LR-12170).

Bite force measurements

Bite force was measured using a piezoelectric force transducer (Herrel et al., 1999; Aguirre et al., 2002). Measurements were performed prior to PCR amplification, avoiding user-behaviour bias, and by a single user, to avoid inter-user error. Each mouse bit three times in a row and the highest bite force was selected for analysis. Group means were compared using Tukey's HSD test. The mice were then euthanized by CO₂ inhalation, and kept at –20°C until further treatment.

Morphometric geometrics and statistical analyses

All skulls were cleaned manually and kept in the collections of Montpellier University. Eleven landmarks were digitized on the

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dorsal side of the skull, and 16 on the mandible using the software tpsDig2.0 (Fig. 1A,B). Landmark data were imported and analyzed in R (R Foundation for Statistical Computing, Vienna, Austria), using the procrustes superimposition method, with functions from Claude (2008). Missing landmarks (owing to the fragility of the mandible), if any, were estimated using a tps

interpolation. Centroid sizes of the skulls and mandibles were computed and used as the size variables (Bookstein, 1991). Principal component analyses (PCAs) were run on superimposed coordinates to check for shape differences between males, XX females and X*Y females. In all analyses, the PC axes retained represented 90% of shape variation. A type II ANOVA was run

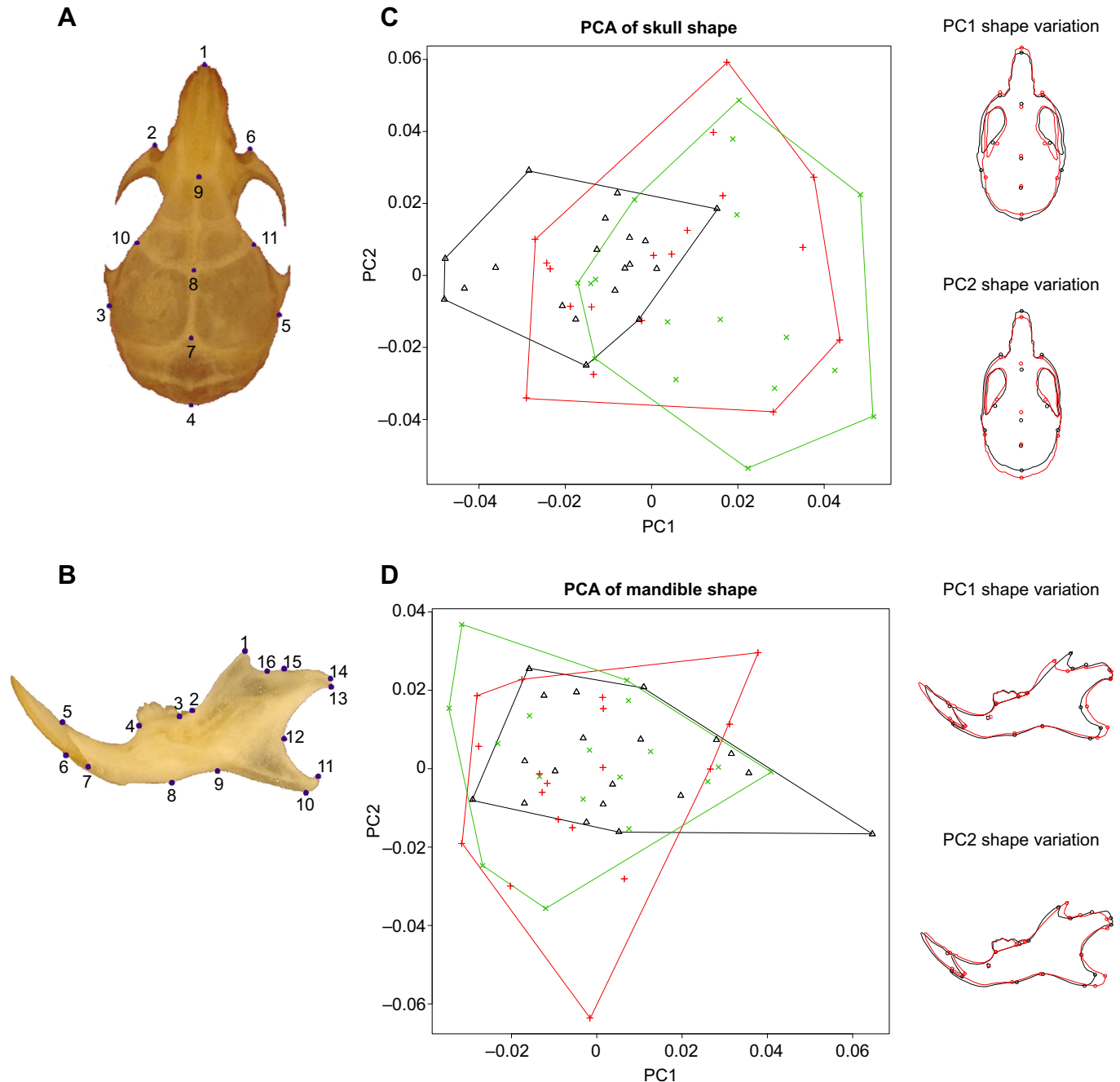


Fig. 1. Geometric morphometric analyses of the skull and mandible of the males, XX females and X*Y females of *Mus minutoides*. Landmarks for (A) the skull and (B) the mandible of *Mus minutoides*. (C) PCA based on the landmark data for the skull, with PC1 representing 16.4% and PC2, 15.3% of the variation. (D) PCA for the mandible, with 10.8% and 9% of the variation displayed by PC1 and PC2, respectively. Black triangles are males ($n=19$), green crosses are X*Y females ($n=17$) and red plus signs are XX females ($n=18$). Skull and mandible shapes on the right of the graph represent minimal (black) and maximal (red) shapes along the PC axes. No significant shape differences were found between these groups. For the skull, positive values on PC1 represent a narrower skull with a longer snout but a shorter braincase, while negative values represent a broader skull with a shorter snout and a longer braincase. On PC2, positive values have a shorter snout and a wider inter-orbital region in comparison to negative values. For the mandible, positive values on PC1 correspond to overall flatter mandibles, with the angular process more posteriorly developed, while the coronoid process is shorter and in a more anterior position than for negative values. On PC2, positive values correspond to mandibles with a shorter molar row, more posterior coronoid process, shorter condylar process and longer angular process than negative values.

Table 1. Results of the ANOVA and MANOVAs run on bite force and morphology of *Mus minutoides*

ANOVA	Factor	SS	d.f.	F	P		
Bite force~Age+Size+Genotype+ Age:Genotype+Size:Genotype	Age	1.15	1	0.0915	0.76		
	Size	133.46	1	10.6033	<0.01		
	Genotype	32.26	2	1.2817	0.29		
	Age:Genotype	35.01	2	1.3907	0.26		
	Size:Genotype	46.17	2	1.8343	0.17		
	Residuals	528.63	42				
Skull MANOVAs		d.f.	Pillai	Approx. F	d.f.	den. d.f.	P
Shape~Size+Genotype×Bite force	Size	1	0.75292	4.6512	19	29	<0.001
	Genotype	2	0.88973	1.2653	38	60	0.20
	Bite force	1	0.40731	1.0489	19	29	0.44
	Genotype:Bite force	2	0.69426	0.8395	38	60	0.71
	Shape~Size×Genotype	Size	1	0.73743	4.4344	19	30
Shape~Size×Genotype	Genotype	2	0.85127	1.2091	38	62	0.25
	Size:Genotype	2	0.79094	1.0673	38	62	0.40
	Shape~Genotype+Size	Genotype	2	0.83306	1.2399	38	66
Shape~Genotype+Size	Size	1	0.70173	3.9624	19	32	<0.001
	Mandible MANOVAs		d.f.	Pillai	Approx. F	d.f.	den. d.f.
Shape~Size+Genotype×Bite force	Size	1	0.77051	5.1247	19	29	<0.001
	Genotype	2	0.84372	1.1521	38	60	0.31
	Bite force	1	0.48403	1.4318	19	29	0.19
	Genotype:Bite force	2	0.81584	1.0878	38	60	0.38
	Shape~Size×Genotype	Size	1	0.75837	4.9555	19	30
Shape~Size×Genotype	Genotype	2	0.80526	1.0997	38	62	0.36
	Size:Genotype	2	0.77411	1.0303	38	62	0.45
	Shape~Genotype+Size	Genotype	2	0.82901	1.2296	38	66
Shape~Genotype+Size	Size	1	0.74118	4.823	19	32	<0.001

The Genotype factor refers to the chromosomal group: XY males, XX females or X*Y females.

to test the effects of sex chromosomal group, age, size and their interactions on bite force. For shape, several models were fitted with a different sum of squares partitioning (Table 1), and MANOVAs were used to assess the influence of sex

chromosomal groups on the principal components of shape variation.

RESULTS AND DISCUSSION

X*Y females bit significantly harder than other females (Fig. 2; data are available on request from the corresponding author). They also bit harder than males, but not significantly so; bite force was similar between males and XX females. Table 2 shows the results of the Tukey’s HSD test.

No major morphological differences appeared between the three groups, which overlapped in morphospace (Fig. 1C,D, Table 1).

X*Y females displayed a significantly greater centroid size than males and XX females (Table 2), even independently of age (Table 1). MANOVAs did not reveal any effect of the group on shape; however, they showed significant allometry shared by all groups (Table 1).

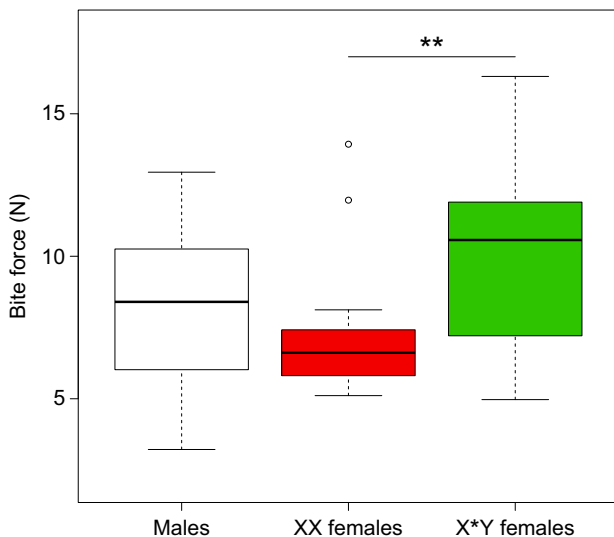


Fig. 2. Boxplot of bite forces of the three *M. minutoides* groups. Data are shown for males (n=19), XX females (n=18) and X*Y females (n=17). Limits of the boxes represent the upper and lower quartiles, the bar is the median, the whiskers represent the maximum and minimum value, or 1.5 times the interquartile distance in cases where outliers are present, and the circles are outliers. Asterisks indicate significant differences between means. Tukey’s HSD test revealed a significant increase in bite force in X*Y females compared with XX females (P<0.01).

Table 2. Results of Tukey’s HSD test for differences in bite force, mandible centroid size and skull centroid size

	Difference	Lower	Upper	P
Bite force				
♂ vs XX ♀	-0.86	-3.05	1.34	0.62
X*Y ♀ vs ♂	1.92	-0.308	4.15	0.1
X*Y ♀ vs XX ♀	2.78	0.52	5.03	0.01
Mandible centroid size				
♂ vs XX ♀	3.15	-20.86	27.16	0.95
X*Y ♀ vs ♂	31.7	7.33	56.07	0.01
X*Y ♀ vs XX ♀	28.55	3.86	53.24	0.02
Skull centroid size				
♂ vs XX ♀	-4.53	-41.21	32.14	0.95
X*Y ♀ vs ♂	67.56	30.33	104.78	<0.01
X*Y ♀ vs XX ♀	72.09	34.38	109.8	<0.01

There was a significant size–bite force correlation ($r \approx 0.6$, d.f.=52, $P < 0.01$ for both bones) when considering the data as a whole (i.e. not taking the groups into account). A relationship was also found between age and bite force ($r = 0.3$, d.f.=49, $P < 0.05$). In the linear model created, the type II ANOVA revealed that, once age and size were taken into account, bite force differences were no longer significant between the groups ($F = 1.28$, d.f.=2, den. d.f.=42, $P > 0.2$), although the tendency for X*Y females to have higher bite force than XX remained. In this model, age had no significant effect, and size remained the main explanatory variable.

We showed that in the African pygmy mouse, X*Y females bite harder than the XX females and, more surprisingly, also bite harder than males (although not significantly so). Limited shape variation between the sex chromosomal groups (Fig. 1C,D) shows that skull and mandible shape are not at the root of the higher bite force of X*Y females. It also suggests that the presence of the Y and/or X* chromosomes does not significantly influence shape. However, without considering the groups, we found a positive relationship between size and bite force, as shown in previous studies (Herrel et al., 2001; Lailvaux et al., 2004; Freeman and Lemen, 2008). Furthermore, X*Y females displayed a greater skull size, which appears to explain part of their increased bite force (Tables 1, 2). Performance changes independent of size but owing to behavioural differences between the groups are subtle and not significant, suggesting that aggressiveness has a much lower impact on performance variation than morphological determinants (here size).

Hormones are also known to influence performance, as demonstrated by hormonal and molecular screening of elite female athletes, which detected sex development disorders with hyperandrogenism (e.g. XY females) in an occurrence 200 times greater than in the general population (e.g. Fénichel et al., 2013). In our model, however, the *M. minutoides* X*Y females harbour typical ovarian anatomy (no ovotestes) and a normal anogenital distance, suggesting a low level of circulating androgen (Rahmoun et al., 2014), observations that have been recently confirmed by preliminary hormonal assays on testosterone (F.V., unpublished data). Therefore, the increased skull size of X*Y females appears to be the main cause of their higher bite force.

Although not much is known about the ecology of *M. minutoides* (Britton-Davidian et al., 2012), the advantages of having higher performance may be twofold. Higher bite force may increase the range of available food resources (Aguirre et al., 2003) by allowing the X*Y individuals to feed on harder food. Second, higher aggressiveness coupled with stronger bites may have a role in intra-sex competition: X*Y females may increase their reproductive success by taking dominance over other females via attacks (Lailvaux and Irschick, 2006). This has been shown for lizards, in which dominance, territorial defense and the outcome of fights are all linked to performance (Herrel et al., 2010; Lailvaux et al., 2004).

Chromosomal changes in the X*Y females may thus give them a dominant position, consistent with the hypothesis that an increase in fitness explains the maintenance of these females, despite the costly loss of the unviable YY embryos (Saunders et al., 2014). Considering our results and those of Saunders et al. (2014, 2016), it appears that the X*Y females obtain selective advantages through many aspects of their fitness: reproductive success, behaviour and whole-organism performance. The effect of the X* chromosome thus goes well beyond feminization, and is at the origin of a complex and multi-factorial X* syndrome that extends to life history traits, behaviour (Saunders et al., 2014, 2016), size variation and performance (present study). Interestingly, these X*Y females were characterized as ‘super females’ because they were shown to

have a better reproductive output than the XX females (Saunders et al., 2014), but in addition, they present hyper-masculinized traits with an enhanced aggressiveness, larger skulls and stronger bite forces than males. It is notoriously hard to assign performance modifications to naturally occurring genetic changes, but this model offers a fantastic framework with which to further connect performance, morphology, behaviour and sex chromosomes.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.G., J.C., F.V.; Methodology: S.G., J.C., J.P., F.V.; Formal analysis: S.G., J.C.; Investigation: S.G., J.P., F.V.; Resources: S.G., J.C., F.V.; Writing - original draft: S.G.; Writing - review & editing: S.G., J.C., F.V.; Supervision: J.C., F.V.

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