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Brief communication

Transmission of the polymorphic dorsal pattern of the Iberian painted frog *Discoglossus galganoi* is compatible with simple Mendelian inheritance

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Abstract

Transmission of the polymorphic dorsal pattern of the Iberian painted frog Discoglossus galganoi *is compatible with simple Mendelian inheritance.* To understand the evolutionary processes involved in the expression of ecologically relevant phenotypical variability it is necessary to disentangle the processes that govern the heredity of said traits. Such is the case of dorsal patterns in the Iberian painted frog, *Discoglossus galganoi*, which presents both a striped and a spotted morph. Here I studied whether the expression of such patterns is subjected to single-locus Mendelian heredity. I crossed males and females with each pattern in all possible combinations and calculated the percentage of each pattern in the offspring. The proportions obtained were compatible with single-locus Mendelian heredity.

Key words: Genetic inheritance, Recessive allele, Dominant allele, Polymorphism, Anuran

Resumen

La transmisión del patrón dorsal polimórfico del sapillo pintojo ibérico Discoglossus galganoi *es compatible con el modelo de herencia mendeliana simple.* Comprender los procesos evolutivos que intervienen en la expresión de la variabilidad fenotípica de importancia ecológica requiere desentrañar el proceso que rige la herencia de dichos rasgos. Tal es el caso de los patrones dorsales del sapillo pintojo ibérico, *Discoglossus galganoi*, que presenta un morfo rayado y otro moteado. En este trabajo se estudió si la expresión de dichos patrones se ajusta al modelo de herencia mendeliana simple. Para ello, se cruzaron machos y hembras en todas las combinaciones posibles de patrones y se calculó el porcentaje de cada patrón en la descendencia. Las proporciones obtenidas fueron compatibles con el modelo de herencia mendeliana simple.

Palabras clave: Herencia genética, Alelo recesivo, Alelo dominante, Polimorfismo, Anuro

The foundations of fields such as evolutionary biology sit on trait heredity (Bonduriansky and Day 2018). Therefore, disentangling the mechanisms that determine the genetic inheritance of traits is essential to understand the evolutionary processes leading to today's biodiversity (Verma 2016). Various inheritance patterns are known to science. Such patterns range from single-locus Mendelian inheritance –where dominant alleles are expressed suppressing the expression of others while recessive alleles are only expressed in the absence of the former (Schacherer 2016)– to a wide array of complex non-Mendelian patterns encompassing single-locus phenomena such as multi-allele and codominance systems and multi-locus processes such as multigenic traits and epistasis (Kenger 2022).

Inheritance patterns of certain traits can be of particular interest. Such is the case of phenotypic polymorphisms, which may be driven by diverse genetic bases (Leimar 2009). Given that polymorphisms involve distinct traits that remain unmixed across generations in conspecifics coexisting within populations, they are bound to entail multi-level evolutionary and population consequences (Forsman et al 2008).

The Iberian painted frog, *Discoglossus galganoi* (Anura, Alytidae), is an example of phenotypic poly-

Fig. 1. Striped (A) and spotted (B) *Discoglossus galganoi* individuals.

Fig. 1. *Individuo rayado (A) y moteado (B) de* Discoglossus galganoi.

morphism. It inhabits a variety of habitats in western and central longitudes in the Iberian Peninsula (Martínez-Solano 2014). Its dorsal coloration is highly variable; brownish, yellowish, pinkish, reddish, or blackish hues may be found, even in the same individual (Martínez-Solano 2014). However, in both sexes, these colors are distributed conforming to one of two patterns: 1) striped frogs, which have one vertebral and two dorsolateral longitudinal bands that are lighter than the background color (fig. 1A); and 2) spotted frogs, which have a relatively uniform background color with patches of other hues that can vary in number and extension (fig. 1B) (Martínez-Solano 2014). In the wild, most populations consist of a mix of striped and spotted individuals that coexist and interbreed (Martínez-Solano 2014), thus meeting the definition of morphs (Huxley 1955).

In a closely related species, *D. pictus*, which shares said traits, there is evidence of Mendelian inheritance with two alleles, with the striped morph being determined by a dominant allele and the spotted morph being determined by a recessive allele (Vences 2012a and references therein). Other works suggest a similar process in *D. galganoi* (Vences 1995, 2012b and references therein). The aim of the present study was to examine whether the expression of these two dorsal patterns is compatible with single-locus Mendelian inheritance in *D. galganoi*. To that end, before the mating season, in November 2019, I collected 12 spotted males, 12 spotted females, 12 striped males and 12 striped females in Pinares de Cartaya (SW Spain; 37° 21' N, 7° 11' O). The animals were captured by hand while active on rainy nights. Following capture, they were allotted to one of four outdoor enclosures. These enclosures were adjacent 6 x 6 m squares with a 1-m brick wall covered by a 1-m steel mesh (5-mm mesh size). The upper parts of these enclosures were also delimited by this mesh. The frogs were thus unable to exit the enclosure and predators were unable to enter. Nonetheless, the invertebrates these frogs feed on could freely enter the enclosures. Each enclosure included an 11-m^2 pond (50 cm deep) where the frogs could reproduce, and a large area of shelter consisting of natural cork plaques. Twelve frogs were housed in each of these enclosures. Each enclosure thus contained: 1) six striped females and six striped males; 2) six striped females and six spotted males; 3) six spotted females and six striped males; and 4) six spotted females and six spotted males. Each enclosure was visually inspected daily and egg

Table 1. Phenotypes of the parentals of the clutches obtained, with the number and percentage of resulting froglets phenotypically striped or spotted in each case, the percentage of each morph expected, and the *P*-value of the Fisher test applied to the observed vs expected percentages.

Tabla 1. *Fenotipos de los progenitores de las puestas obtenidas, con el número y porcentaje de saltones fenotípicamente rayados o moteados en cada caso, el porcentaje esperado de cada morfo, y el valor* P *del test de Fisher aplicado a los porcentajes observados y los esperados.*

Male	Female	Striped n	Spotted n	Observed striped %	Spotted %	Striped %	Expected spotted %	Fisher test *P*-value
Spotted	Spotted	0	478	0.00	100.00	0.00	100.00	NA
Spotted	Spotted	0	236	0.00	100.00	0.00	100.00	NA
Striped	Striped	302	0	100.00	0.00	100.00	0.00	NA
Striped	Striped	61	34	64.21	35.79	75	25	0.156
Striped	Striped	198	58	77.34	22.66	75	25	0.604
Striped	Striped	56	20	73.68	26.32	75	25	1.000
Striped	Striped	206	77	72.79	27.21	75	25	0.633
Striped	Striped	121	37	76.58	23.42	75	25	0.793
Spotted	Striped	222	239	48.16	51.84	50	50	0.598
Spotted	Striped	202	165	55.04	44.96	50	50	0.184
Striped	Spotted	230	226	50.44	49.56	50	50	0.947
Striped	Spotted	147	138	51.58	48.42	50	50	0.738

masses detected (12 in total) were transferred to the laboratory. The minimum span between the laying of two consecutive clutches within the same enclosure was three days, so the probability of scramble competition leading to multiple paternity of each individual egg mass was low. The resultant tadpoles were raised in a 6-litre aquaria that contained a maximum of 50 individuals. The water was fully changed twice a week, and food was provided ad libitum in the form of boiled spinach. At the end of the metamorphosis, when the dorsal pattern is expressed, I counted the number of striped and spotted individuals resulting from each egg mass, and calculated the percentage of striped and spotted individuals in each egg mass (table 1). A Fisher test was then run to test whether the percentages observed differed from the percentages expected in a Mendelian inheritance context (table 1).

The egg masses resulting from spotted parents yielded 100 % of spotted offspring (table 1). This is compatible with the parents being homozygotes for hypothetical autosomal recessive alleles determining the spotted phenotype.

Among the egg masses resulting from striped parents, one yielded 100 % of striped offspring, while 5 yielded approximately 75 % striped and 25 % spotted individuals (table 1). One of these differed from these percentages (64.21 striped, 35.79 spotted; table 1) but could be a consequence of the small size of this egg mass. The former is compatible with at least one of the parents being a homozygote for a hypothetical dominant allele determining the striped pattern. The remnant cases are compatible with both parents being heterozygotic for the dominant and the recessive alleles determining the striped and the spotted phenotypes respectively.

The egg masses resulting from the combination of spotted and striped parents yielded approximately 50 % of spotted and 50 % of striped offspring, irrespective of the sex which carried each pattern (table 1). These cases are compatible with spotted parents being homozygotes for the recessive allele determining the spotted patterns and striped parents being heterozygotes.

These results thus suggest that the phenotypical expression of the dorsal pattern of this species is subject to simple Mendelian inheritance. Two alleles might be involved: the spotted pattern could be determined by a recessive allele, whereas the striped pattern could be determined by a dominant allele. Other studies have also found dorsal pattern polymorphisms in anurans to be consistent with single-locus inheritance, such as *Dendrobates pumilio* (Summers et al 2004) or *Eleutherodactylus coqui* (O'Neill and Beard 2010), among others.

These results also suggest that dominant homozygotes were under-represented among the parents. This could be a consequence of the striped pattern being considerably less abundant, representing around 30 % of the population in this study system (Zamora-Camacho and Aragón 2022). The spotted pattern is typically dominant in populations of *D. galganoi* (Vences 1995, 2012b and references therein) and *D. pictus* (Vences, 2012a and references therein). These differences in abundance could follow disparities in predation pressure. Indeed, plasticine models mimicking striped frogs receive more predator attacks than their spotted counterparts. This observation is aligned with the fact that spotted individuals allow a potential predator to approach them more easily than striped individuals before they start to flee (Zamora-Camacho and Aragón 2022).

These data, however, should be taken with caution, first because the number of egg masses obtained was limited. Furthermore, given the experimental design, I was unable to determine precisely which parents produced each egg mass. Ideally, these problems would have been solved by isolating each pair in a container until oviposition, but this species does not reproduce in artificial conditions. The animals therefore had to be allocated in large outdoor facilities that imitate natural habitats. This represented a major logistic limitation. Moreover, given the prolonged developmental period until maturity, backcross breeding was unrealistic, as it would have involved maintaining the parents in captivity for several years, while the average lifespan of this species is around 4 years (Esteban et al 1998).

References

Bonduriansky R, Day T, 2018. *Extended heredity: a new understanding of inheritance and evolution*. Princeton University Press, Princeton, New Jersey.

Esteban M, Castanet J, Sanchiz B, 1998. Inferring age and growth from remains of fossil and predated recent anurans: a test case using skeletochronology. *Canadian Journal of Zoology* 76, 1689-1695.

Forsman A, Ahnesjö J, Caesar S, Karlsson M, 2008. A model of ecological and evolutionary consequences of color polymorphism. *Ecology* 89, 34-40. DOI: 10.1890/07-0572.1

Huxley J, 1955. Morphism and evolution. *Heredity* 9, 1-52.

Kenger IH, 2022. Non-Mendelian inheritance. In: *Health and Science 2022-III*, 109-118 (H. Kaya, Ed). EFE Academy Publishing, Istanbul, Turkey.

Leimar O, 2009. Environmental and genetic cues in the evolution of phenotypic polymorphism. *Evolutionary Ecology* 23, 125-135. DOI: 10.1007/s10682-007-9194-4

Martínez-Solano Í, 2014. Sapillo pintojo ibérico–*Discoglossus galganoi*. In: *Enciclopedia Virtual de los Vertebrados Españoles* (A Salvador, Í Martínez-Solano, Eds). Museo Nacional de Ciencias Naturales, Madrid, www.vertebradosibericos.org

O'Neill EM, Beard KH, 2010. Genetic basis of a color pattern polymorphism in the coqui frog *Eleutherodactylus coqui*. *Journal of Heredity* 101(6), 703-709. DOI: 10.1093/jhered/esq082

Schacherer J, 2016. Beyond the simplicity of Mendelian inheritance. *Comptes Rendus Biologies* 339, 284-288. DOI: 10.1016/j.crvi.2016.04.006

Summers K, Cronin TW, Kennedy T, 2004. Cross-breeding of distinct color morphs of the strawberry poison frog (*Dendrobates pumilio*) from the Bocas del Toro Archipelago, Panama. *Journal of Herpetology* 38, 1-8, https://www.jstor.org/stable/1566079

Vences M, 1995. Erste Untersuchungen über die Fortpflanzungsbiologie des Iberischen Scheibenzünglers *Discoglossus galganoi* (Amphibia: Anura: Discoglossidae). *Acta Biologica Benrodis* 6, 89-98.

Vences M, 2012a. *Discoglossus pictus* Otth, 1837-Gemalter Scheibenzüngler. In: *Handbuch der Amphibien und Reptilien Europas. Froschlurche I*, 225-248-211 (K Grossenbacher, Ed). Aula Verlag, Wiebelsheim.

Vences M, 2012b. *Discoglossus galganoi* Capula, Nascetti, Lanza, Bullini und Crespo, 1985-Iberischer Scheibenzüngler. In: *Handbuch der Amphibien und Reptilien Europas. Froschlurche I*: 187-211 (K Grossenbacher, Ed). Aula Verlag, Wiebelsheim.

Verma AK, 2016. Biodiversity: its different levels and values. *International Journal on Environmental Sciences* 7, 143-145.

Zamora-Camacho F J, Aragón P, 2022. Antipredator responses of the morphs of an amphibian species match their differential predation pressures. *Behavioral Ecology and Sociobiology* 76, 26. DOI: 10.1007/s00265-022-03140-6

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