NATURAL HISTORY NOTE

Sex-specific ventral dichromatism and melanization in harlequin toads (*Atelopus*): a common but overlooked character of unknown function

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Abstract

Hidden colors are a widespread phenomenon in the animal kingdom, particularly in anurans. In some cases, hidden colors are suddenly exposed during defensive displays to startle predators, others seemingly remain hidden—particularly from researchers. Amazonian species of Neotropical harlequin toads (genus *Atelopus*) show striking and consistent ventral sexual dichromatism where females show and males generally lack melanization. Inspired by these observations we undertook a deeper inquiry across this species-rich genus. We collected data on ventral sexual dichromatism in *Atelopus* species and scored expression of sex-specific ventral melanization (i.e. black, brown and/or grey coloration). Ventral sexual dichromatism was present throughout the entire range of the genus and in almost all phylogenetic groups. However, there was a clear geographic signal with this trait being most common and widespread in Amazonian *Atelopus* species. Ventral melanization was correlated with temperature and elevation. Focusing on the Amazonian species, we present hypotheses on potential functions of sexually dimorphic ventral patterns and sex-specific ventral melanization as a baseline to further investigate the dynamics of sexual and natural selection as potential drivers of these traits. Selective pressures on less exposed body parts, such as ventral sides, likely differ considerably from those on dorsal appearance. Given the amount of research on amphibian coloration, it is remarkable how little we know about the evolution, function and underlying mechanisms of ventral appearance. We hope our work will spark more interest in the flip side of amphibians, thereby broadening our understanding of animal coloration.

Keywords Hidden colors · Aposematism · Sexual selection · Sexual color dimorphism · Natural selection

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Introduction

Colors and patterns in amphibians are incredibly diverse and can serve multiple functions including intra- and interspecific communication, protection from solar radiation as well as thermoregulation (Duellman and Trueb 1986; Wells 2007) and as such are subject to both natural and sexual selection. Many anurans such as Neotropical poison frogs (Dendrobatidae) and Malagasy mantella frogs (Mantellidae) are well-studied for their striking (aposematic) coloration deterring potential predators (Summers et al. 2015; Rojas 2016). Other anurans are known for rather cryptic coloration by background-matching or disruption, such as Neotropical dead-leaf pattern toads, genus *Rhinella*, or Paleotropical mossy frogs, genus *Theloderma* (Toledo and Haddad 2009; Barnett et al. 2021). Moreover, sexual dichromatism, where males and females express different coloration, is widespread among anuran species (Bell et al. 2017). Classic examples include the temporary color changes of male moor frog (*Rana arvalis*) during the breeding season or the ontogenetic color changes in *Rhinella icterica* with dichromatism appearing at sexual maturity (Bell and Zamudio 2012; Sztatecsny et al. 2012).

The dorsal coloration in frogs and toads is most frequently studied, while the function and evolution of ventral colors and patterns are less well understood, even though they are often equally as striking and complex (cf. Wells 2007; Toledo and Haddad 2009; Loeffler-Henry et al. 2023). Ventral appearance is likely to be under different selective pressures compared to dorsal sides due to its concealed nature, being less exposed to environmental pressure, predators, conspecifics (and researchers).

Ventral coloration is mostly studied in the context of anti-predatory deimatic displays (Bajger 1980; Kariş et al. 2017). For example, the chemically defended fire-bellied toads in the genus *Bombina* and the Neotropical bufonid genus *Melanophryniscus*, expose their conspicuous ventral coloration during a defensive behavior called the *unken reflex* (Grant et al. 2012; Bordignon et al. 2018). In contrast, the influence of environmental variables on ventral coloration are less researched, although both color perception and predator communities may change along environmental gradients.

However, the role of ventral coloration in anurans is probably more complex. An example is the species-rich genus of harlequin toads (*Atelopus*), a group of toxic anurans from Central and South America in which an *unken reflex* has been observed only occasionally (e.g. *A. exiguus*). In many *Atelopus* species colorful ventral sides have been described (Lötters 1996; Coloma et al. 2000; *Atelopus* sp. "wampukrum" in Fig. 1), including patterns of sexual dichromatism: females commonly present dark blotches on the ventral side while males usually entirely lack ventral melanization (Fig. 1), as noted in *Atelopus pulcher, A. loettersi* and *A. manauensis* (Lötters et al. 2002; De la Riva et al. 2011; Jorge et al. 2020). In contrast, the reversed pattern, with only males being ventrally melanized, is found in species from the Colombian Andes, e.g., in *A. nocturnus* and *A. subornatus* (Lötters 1992; Bravo-Valencia and Rivera-Correa 2011). Equally, some *Atelopus* species are sexually monomorphic with either no melanized patterns or patterns that are similar between males and females, such as *A. ardila* where the venter is uniform cream in males and females (Coloma et al. 2010).

Here, we record the presence/absence of ventral sexual dichromatism and specifically the sex-specific differences of ventral melanization in the genus *Atelopus* and explore how ventral dichromatism is linked to geographic distribution and species-specific environmen-

Fig. 1 Ventral sexual dichromatism in species of the genus *Atelopus*, males (right) and females (left). Top, *Atelopus spumarius* sensu stricto from the upper Amazon basin showing ventral sexual dichromatism in both color and pattern; middle, *Atelopus* cf. *sonsonensis* Pensylvania (sp. 4 sensu Rueda-Almonacid et al. 2005) from the Colombian Cordillera Central showing ventral melanization in males only; bottom: *Atelopus* sp. *"wampukrum"* from the Cordillera del Condor, an example for striking ventral sexual dichromatism of coloration, showing melanization in females only. Figure not to scale. Photos: J. Culebras and C. Heine

tal variables. Further, we develop hypotheses exploring potential adaptive functions of sex-specific ventral coloration.

Methods

We opportunistically extracted information from species descriptions, by visual examination of available photographs of live or preserved specimens, own observations and scientific collections to assess presence/absence of sexual dichromatism based on either differences in melanization or other colors between males and females or both. We refrained from using image processing software for refined quantification due to the lack of comparability with data necessarily obtained by eye (i.e. species descriptions, field observations). As sex-specific differences in melanization appeared to be the most common reason for dichromatism, we further scored whether males, females both or neither expressed ventral melanization (Supplementary Table S1). In total, we used information from 131 described and undescribed *Atelopus* species (Lötters et al. 2023; Supplementary Table S1). Species were scored based on visual examination as soon as data on a minimum of one individual per sex was available to us, particularly because several *Atelopus* species are known from few specimens only (e.g. *A. orcesi;* Coloma et al. 2010). Ventral sexual dichromatism, which was assessed as presence/absence data, was considered present for all species that showed distinct patterns and/or colors between sexes on at least one third of their ventral surface in all examined specimens of at least one population (we observed within-species variation in the presence/absence of this trait in *A. limosus* only, a species that comprises two distinct morphotypes). Sex-specific ventral melanization was scored as present, when all examined individuals of at least one population presented at least about 10% of the venter covered in dark pattern (black, brown and/or grey blotches, dots, lines or markings). When such melanization was present, we further examined if it was observed in male or female individuals only, or if it occurs in both sexes (at least one specimen of each sex). Information for at least one adult male and female were available from 94 species regarding ventral sexual dichromatism and from 96 regarding ventral melanization. To explore whether there are correlations of environmental data with the presence/absence of ventral dichromatism and melanization, we derived location-specific environmental data using ArcGIS Pro (ESRI). This was based on 596 coordinates for 105 of the 131 species after merging sites with a distance of < 30 arc seconds to others of the same taxon. For 60 species, information from more than one population was available (63.8% of the 94 species known from >1 locality; Lötters et al. 2023). We used the bioclimatic variable 'mean annual temperature' (Bio1) from the CHELSA TraCE21k data (Karger et al. 2023), and elevation from the World Elevation Terrain digital terrain model (ESRI). All data, sample sizes and data sources are provided in Supplementary Table S1. We have intentionally not published locality data from this study as doing so could threaten populations due to illegal collecting.

We fitted Generalized Additive Models (GAMs) in R 4.3.0 (R Core Team 2021) using the package *data.table* (Dowle and Srinivasan 2022) to load data, *mgcv* (Wood 2011, 2017) for modelling and *itsadug* (van Rij et al. 2022) to inspect the results. We visualized results in *ggplot2* (Wickham 2009). We generated separate models for the dependent variables ventral sexual dichromatism and ventral melanization. Ventral melanization was further modelled separately for each sex as well as together to account for the differences between sexes.

Smooth predictors included locality (latitude and longitude), average temperature, elevation, and the interaction between the last two as a tensor term. All models had a binomial error structure. Since there was almost no variation in temperature and elevation for populations from the Amazon basin and the Guianas, we removed these observations (any observation E of 65° W) focusing on the mountain ranges and upper Amazon to elucidate any potential effects of temperature and elevation on ventral sexual dichromatism as well as on ventral melanization. The code as well as detailed model results and supplementary model visualization are provided in Supplementary Data D1.

Results

We identified 26 *Atelopus* species with ventral sexual dichromatism (27.7% of the 94 species with sufficient data on this trait; Fig. 2; Supplementary Table S1). In 41 species, ventral melanization was present in at least one sex (42.7% of the 96 species with sufficient data on this trait; Fig. 3; Supplementary Table S1). Of these, in 12 only females and in five only males possessed ventral melanization. Ventral sexual dichromatism occurred throughout almost all major clades, i.e. the *carrikeri*, the Andean, the *flavescens-spumarius*, the *tri-*
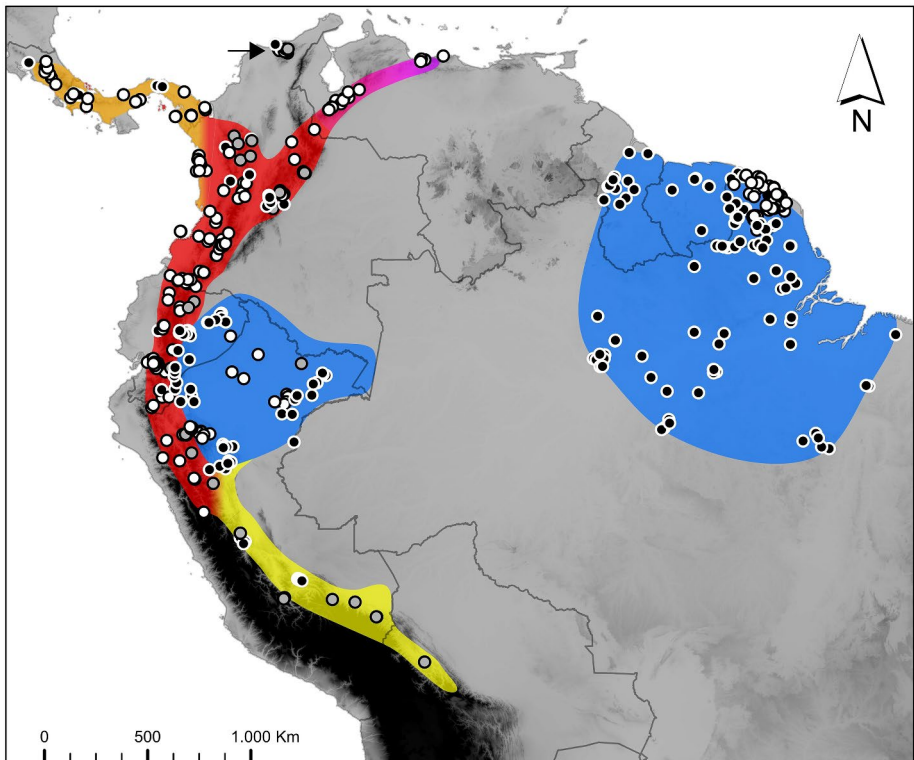

Fig. 2 Spatial distribution of ventral sexual dichromatism in the genus *Atelopus*: black dots=present, white dots=absent, grey dots=unknown. Colored polygons indicate the approximate ranges of phylogenetic clades: red=Andean clade, pink=cruciger clade, blue=*flavescens-spumarius* clade, yellow=*tricolor* clade, orange=*varius* clade; the *carrikeri* clade indicated by an arrow
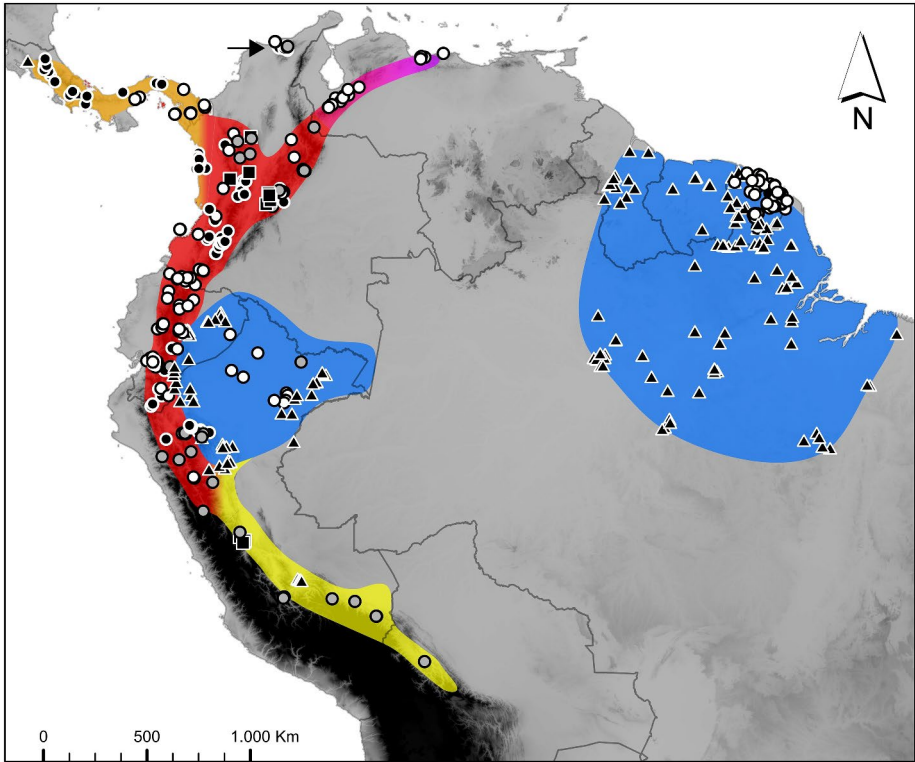
Fig. 3 Spatial distribution of sex-specific ventral melanization in the genus *Atelopus*: black dots=present in both sexes, white dots=absent in both sexes, black triangles=present in females only; black squares=present in males only; grey dots=unknown. Colored polygons indicate the approximate ranges of phylogenetic clades: red=Andean clade, pink=*cruciger* clade, blue=*flavescens-spumarius* clade, yellow=*tricolor* clade, orange=*varius* clade; the *carrikeri* clade indicated by an arrow

color, and the *varius* clades (Lötters et al. 2011; Böning et al. 2022). The only exception where this trait was absent in all species is the *cruciger* clade (Fig. 2; Supplementary Table S1).

The GAMs revealed that geographic location was a significant predictor for the presence of ventral dichromatism (Longitude*Latitude: edf=19.002, Ref.df=22.646, $\chi 2$=61.986, $p<0.0001$), while temperature (edf=3.024, Ref.df=3.871, $\chi 2$=6.775, p=0.1256), elevation (edf=3.857, Ref.df=4.864, $\chi 2$=9.447, p=0.0705) and their interaction (edf=1, Ref.df=1, $\chi 2$=0.035, p=0.8509) were not.

Ventral melanization was prominent across all temperatures and elevations, but mainly so for the hottest area of each elevational step. This seems to be the case for females, while for males even though the general pattern appeared similar, the lowest elevations were almost completely devoid of melanization. Specifically, ventral melanization was significantly predicted by geographic location in males (Longitude*Latitude: edf=18.9, Ref.df=22.297, $\chi 2$=69.462, $p<0.0001$) and females (Longitude*Latitude: edf=22.054, Ref.df=25.493, $\chi 2$=80.71, $p<0.0001$). Relationships with temperature and elevation were significant for (i) the interaction of temperature and elevation on male ventral melanization (edf=1, Ref.

df=1.001, $\chi2$=8.544, p=0.00347); (ii) temperature alone on the presence of female ventral melanization (edf=4.112, Ref.df=5.354, $\chi2$=12.584, p=0.0379) and (iii) elevation alone on the presence of female ventral melanization (edf=1, Ref.df=1, $\chi2$=5.591, p=0.0181).

Discussion

Sexual dichromatism of ventral sides was geographically widespread across the distribution of the genus *Atelopus* and was observed in members from all but one clade, including the most basal one (*carrikeri* clade), hinting at this character to potentially be basal in the genus, followed by repeated losses. The geographic signal likely mirrors a phylogenetic signal, as the different clades occur in allopatry or parapatry (Lötters et al. 2011; Böning et al. 2022). However, the most complete published phylogeny covers only few markers and less than one fourth of all species, preventing us from phylogenetic mapping of ventral sexual dichromatism and sex-specific ventral melanization. We therefore encourage such approaches for future studies once a broader molecular sampling has become available.

So far, the function of ventral sexual dichromatism in *Atelopus* is unknown, likewise whether it is shaped by sexual or natural selection or, as seems most likely, by both. Environmental parameters investigated did not explain the presence or absence of ventral dichromatism in this group.

Sex-specific ventral melanization is widespread, implying that it is a highly conserved trait. We found significant relationships of temperature and elevation with ventral melanization. Melanization of ventral sides is consistently prominent in the hottest areas of each elevational step with the exception that males show no melanization in the lowest elevations. Some studies have found evidence that higher melanization is more prevalent in high-altitude species suggesting a role in thermoregulation and protection from UV radiation (Trullas et al. 2007; Alho et al. 2010). The presence of melanization on the ventral side, a much less exposed body part, is difficult to explain in this framework. Although we observed a seeming relationship of ventral melanization with temperature and elevation, we believe both variables do not exclusively explain our observations. However, despite our broad taxon sampling, information is often derived from few individuals and few localities only, particularly in the many poorly known, locally endemic and 'lost' taxa (Supplementary Table S1). This uncertainty regarding within-species variation, as well as the challenges when deriving color information from historic museum specimens and possible inaccuracies of trait characterization when deriving color data opportunistically from different data sources require caution for interpreting our observations.

It is clear from our study that patterns of ventral sexual dichromatism as well as presence of ventral melanization are geographically complex, making it highly unlikely that a single functional explanation can be applied to all populations and species. Consequently, we focus below on the least imperiled and therefore best sampled Amazonian species and the robust sex-specific difference of ventral melanization in this group for a functional discussion.

Sexual dichromatism in anurans commonly implies a function in intraspecific communication such as territoriality (e.g., in the genus *Staurois*; Stangel et al. 2015) or mate-choice (e.g., in *Oophaga pumilio*; Maan and Cummings 2009). However, different colors of males and females can equally be the result of sex-specific life history (Dale et al. 2015). Many *Atelopus*, at least for some periods throughout the year, live along streams in visually and

acoustically complex environments (e.g., Sexton 1958; Dole and Durant 1974; Crump 1988; Lindquist et al. 1996; Ringler et al. 2022). Besides acoustic signaling, visual signaling might therefore be crucial in intraspecific communication for instance with regard to hand and foot signaling (i.e. semaphoring) in *Atelopus* (e.g., Lindquist and Hetherington 1996). Ventral coloration which is exposed during intraspecific interaction, likely has a signaling function in diurnal anurans (Hödl and Amézquita 2001; cf. Starnberger et al. 2014). So far observed in at least some *Atelopus* species, males expose the vocal sac to conspecifics (both male and female) during vocalization in both territorial and reproductive contexts (cf. Lindquist and Hetherington 1996; Supplementary Video 1). Males of Amazonian *Atelopus* species that, at least on anterior parts, commonly lack ventral melanization, can often be observed calling from elevated sites such as tree trunks, increasing the visibility of their ventral surfaces, particularly the throat, to conspecifics. Amazonian *Atelopus* species, however, also present the longest advertisement call durations in the genus (i.e. the 'pulsed call' type: Cocroft et al. 1990; Lötters et a. 2019) and vocalize more frequently than most other *Atelopus*, perhaps implying a stronger selective pressure for bright, non-melanized, throats (A. Plewnia, S. Lötters, unpubl. data).

Consequently, the lack of any melanization on the ventral side could maintain visual signal efficacy during acoustic signaling. We hypothesize that in order to maintain high signal efficacy of throats during vocalization in intra- and or intersexual interactions, melanization of the ventral region may be inhibited in males of certain *Atelopus* species. To test this hypothesis, models of *Atelopus* with inflatable throats (similar to those used for other anurans by James et al. 2022) that can be manipulated in coloration (e.g. yellow vs. yellow with black spots) can be used to run choice experiments testing effects of throat coloration on female preference.

Understanding sex-specific ventral melanization in Amazonian *Atelopus* species requires broader consideration of their life histories. Substantial differences in life history between males and females have been reported in several *Atelopus* species (Dole and Durant 1974; Lötters 1996; Luger et al. 2009; Ringler et al. 2022). In many species, males maintain their territories on stream banks at least throughout parts of the year where they can occur in high densities (Crump 1988; Ringler et al. 2022; Señaris et al. 2023). Females, on the other hand, are scattered throughout the forest farther from streams, returning to their spawning sites only for reproduction (Lindquist et al. 2007; Ringler et al. 2022). These differences in life history and consequently microhabitat, can likely influence the expression of ventral melanization, e.g. due to exposure to distinct predator communities. For instance, ventral melanization may act disruptive to ventral color in complex habitats and particularly to predators that do not perceive colors while enhancing contrast and conspicuousness in uniform environments. Such factors, along with other ecological and evolutionary dynamics, could contribute to the expression of ventral sexual dichromatism in Amazonian *Atelopus*.

Not much is known about predators of *Atelopus*. As far as we are aware, there are only a few documented cases of predation on adult harlequin toads. These include predation by the carunculated caracara (*Phalcoboenus carunculatus*; De Vries et al. 1983), a fire-bellied snake (*Erythrolamprus epinephalus*; Greene 1997; Lindquist et al. 2007), a climbing catfish (*Astroblepus* sp.; Cruz-Garcia et al. 2023), a golden wolf fish (*Hoplerythrinus unitaeniatus*; Lima et al. 2019), a water bug (*Abedus* sp.; González-Maya et al. 2019) and by an unidentified crab (Barrio-Amorós et al. 2021). As far as we are aware, there is only one documented case of predation on a juvenile harlequin toad by an Amazonian white-lipped

frog (*Leptodactylus mystaceus*; Pinto and Costa-Campos 2015). It is interesting that four of these observations document aquatic predators, which are potential visual receivers of ventral coloration. With that in mind, it seems plausible that females of Amazonian *Atelopus* species possess dark blotches, which could render them potentially cryptic to aquatic predators during aquatic phases pre, during and post egg deposition (with the amplectant male on its back). This could explicitly be tested by deploying models of *Atelopus* with different ventral patterns in the water (attached to the shore) and monitor them using camera traps for several weeks.

Ontogenetic change of colors is known to enhance camouflage in juveniles to reduce predation, with development of conspicuous colors or patterns with sexual maturity (Stangel et al. 2015). Ontogenetic color changes from cryptic to conspicuous are known from several harlequin toad species, where dorsal coloration transitions from uniformly greenish and black to bright green, yellow, orange, pink or red with black markings in adults (e.g., *A. balios, A. certus, A. elegans, A. varius, A. zeteki*; Lötters 1996; A. Plewnia, S. Lötters, unpubl. data). Less is known about potentially similar ontogenetic changes of ventral coloration, specifically of melanization. Generally, we suspect that changes in skin toxicity or changes in predator species over the lifetime of these animals could influence changes in color and pattern expression.

So far, ventral appearance in amphibians have gained very little attention, despite the ease of data availability via photographs and field guides. Ventral appearance is usually discussed in the context of defensive behaviors, where hidden colors are revealed during predator encounters (Rudh and Qvarnström 2013; Loeffler-Henry et al. 2023). The steppingstone hypothesis of aposematism evolution (Loeffler-Henry et al. 2023), where conspicuous hidden signals are thought to evolve into overt aposematic signals does not account for the widespread and variable, ventral sexual dichromatism we describe in *Atelopus*, demanding alternative explanations. Based on our observations in *Atelopus* we suspect that ventral sexual dichromatism and sex-specific ventral melanization may be widespread among diurnal anurans that commonly engage in visual signaling with functions in need of explicit testing.

Declarations

Ethical approval Not applicable.

Consent for publication All authors have read and approved the final version of the manuscript.

Competing interests The authors declare no competing interests.

Inclusion and diversity statement The authors greatly value equity, diversity and inclusion (EDI) in science. This study was conducted under consideration of EDI best practice, however, the authors acknowledge the gender bias in the author list. The authors represent all career stages from undergraduate student to full professor. The authors come from Germany, Italy and Brazil. One or more of the authors self-identifies as a member of the LGBTQ+community. We actively worked to promote gender balance (of first author) in our reference list, which, however, we did not fully reach.

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