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Challenging the reliability of dorsal colouration patterns for the long-term identification of *Dendrobates tinctorius* (Cuvier, 1797) poison dart frogs

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Skin colouration patterns are an important trait of animals which is involved in many functions, from communication to thermoregulation (Maan and Cummings, 2008; Singh et al., 2015). Since, for most organisms, skin colouration patterns become stable in adulthood, these can be used for individual identification and reduce the need to intrusively mark individuals (*e.g.*, tags or tattoos) in recapture studies (Schlüpmann and Kupfer, 2009). This identification technique, Individual Identification Pattern Recognition (IIPR), is used across all major vertebrate groups (Hammond et al., 1990; Arzoumanian et al., 2005; Gamble et al., 2008; Sherley et al., 2010; Balaguera-Reina et al., 2020). For each species, it is therefore essential to know whether adult colouration pattern is dynamic or stable to allow the application of these methods in capture-recapture studies. Still, only a few studies have examined this stability throughout adulthood (Morrison et al., 2016; Lunghi et al., 2019).

The Dyeing Dart Frog, *Dendrobates tinctorius* (Cuvier, 1797), is indigenous to the tropical rainforests of South America (Noonan and Gaucher, 2006). This species is characterised by a colourfully patterned epidermis, exhibiting hues from brilliant yellow to deep blue, serving as a visual deterrent to predators given the Dyeing Dart Frog's inherent toxicity (Silverstone, 1975; Summers and Clough, 2001; Comeault and Noonan, 2011). Herpetologists have frequently used IIPR when studying adult *D. tinctorius* (Born et al., 2010; Courtois et al., 2012; Rojas and Pašukonis, 2019). Dorsal colouration patterns change drastically in *D. tinctorius* in the first 18 months after metamorphosis and somewhat stabilise after that (Yuan et al., 2022). While Yuan et al. (2022) did find shifts in captive (potentially inbred) adult *D. tinctorius* colouration, it is unknown which types of changes these are, whether they are also observed in wild individuals, and to what degree this may affect their identification and lead to inaccurate estimates of population dynamics (*e.g.*, survival).

Herein, we investigate changes in the dorsal colouration pattern of wild *D. tinctorius* during adulthood in order to: describe the type of pattern change observed, determine whether there are differences between colour-morphs, and support the appropriateness of IIPR methods for this species. To do so, we tracked dorsal colouration pattern changes (patch appearance, disappearance, or merging), measuring dorsal colouration patterns twice (1–2 years apart) in wild adult individuals.

Materials and Methods

Study subject and housing. *Dendrobates tinctorius* is a highly polymorphic species, displaying variation in skin colouration and body size associated with different geographic regions (Noonan and Gaucher, 2006; Rojas and Pašukonis, 2019). This variation between populations is reflected in distinct colour-morph nomenclature (Wollenberg et al., 2008). We compared the pattern of wild individuals with 1–2 years apart to describe the type of pattern change during this period in wild individuals, to determine whether there are differences between colour-morphs, and to validate the use of IIPR methods in this species.

During 2020 and 2021, we hand-captured (using gloves) 36 adult *D. tinctorius* of three colour-morphs (Nominal (n = 10), Ouanary (n = 16), and Giant orange (n = 10)) at Montagne Sable (4.4768°N, 52.0306°W), Ouanary (4.2078°N, 51.6628°W), and Mataroni

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(4.0740°N, 52.1563°W), respectively (Fig. 1). Life-stage was assessed based on body size, with only above-average individuals being collected; further confirmation was obtained when all individuals reproduced in captivity. Individuals were kept in pairs (a male and a female) according to their colour-morph and maintained in terraria (n = 18) within a greenhouse in the Plateforme d'Écologie Expérimentale Amazonienne (PLEEA) platform in Cayenne, French Guiana. Terraria contained a cork floor, dried leaves from the surrounding forest, a half coconut shell over a petri dish with water, a large piece of lignified angiosperm ramus, and several natural plants. The greenhouse had natural lighting and was moistened with overhead sprinklers twice a day. Individuals were fed three times a week with flightless *D. hydei* dusted with a mix of Repashy® Supercal NoD and Supervite as a nutritional supplement before feeding.

Image sampling and analysis. We photographed each individual in the experiment twice, once upon entering the collection in January–December 2020 and again in November 2021. New patches' appearances, disappearances, or mergers were tracked visually.

IIPR software comparison. In order to determine whether the described pattern changes would affect the ability of IIPR software to match pictures of the same individual, all pictures of the experiment were processed with two IIPR software programs (Wild-ID (Bolger et al., 2012) and I3S pattern + (https://reijns.com/i3s/)). These software programs were used to match each individual's "after" picture to all "before" pictures (across all colour-morphs). The position of the correct match within the top 20 best matches (the most likely pictures to be from the same individual used in the query) was extracted to determine the accuracy of both software programs in individual identification.

Results and Discussion

Wild-caught *Dendrobates tinctorius* individuals were photographed upon entering captivity and re-photographed 1–2 years later. Overall, 42 %

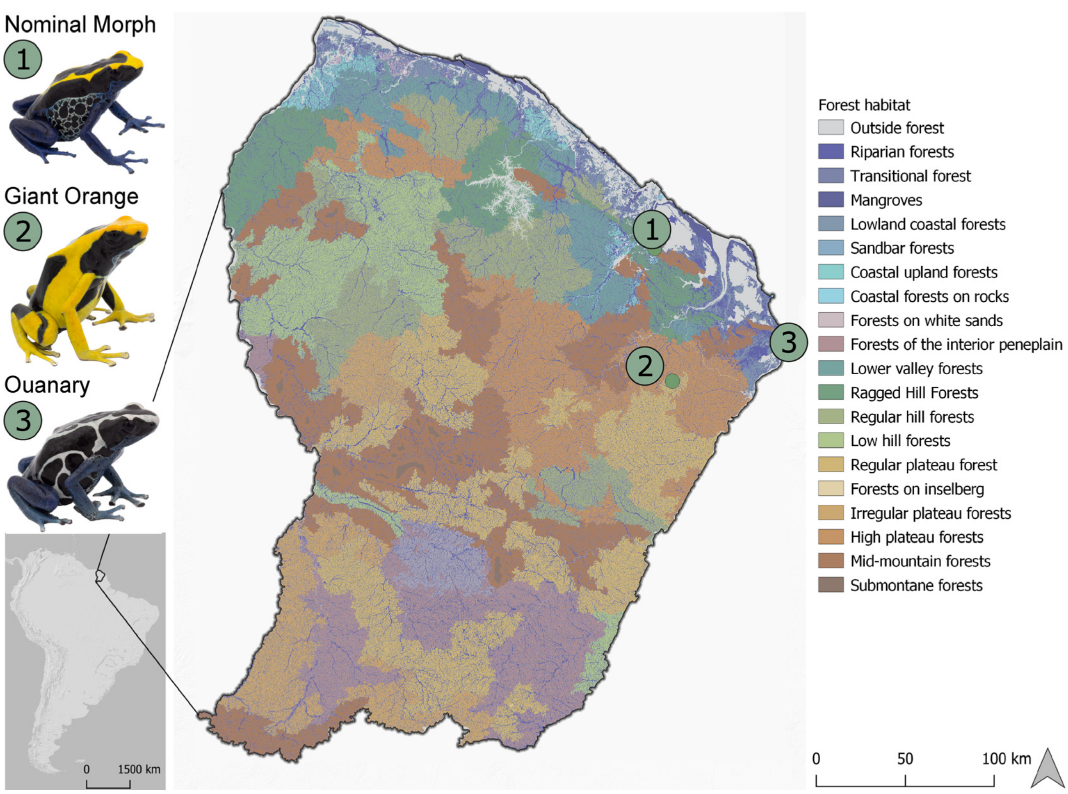

Figure 1. Map of French Guyana with the collection sites of the *Dendrobates tinctorius* colour morphs analysed in this study. Pictures by Ugo Lorioux-Chevalier. Map source: ONF GUYANE, 23 February 2018.

of individuals presented changes in their dorsal colouration pattern (15 out of 36, Fig. 2, Appendix 1). These changes consisted of 1) patch disappearance; 2) patch appearance; and 3) patch split (Fig. 2). The most common changes were patches disappearing or splitting; in a single individual, a new patch was observed (Fig. 2A). No changes in dorsal colouration pattern were observed in individuals belonging to the Nominal morph (Appendix 2); whilst almost all individuals belonging to the Ouanary morph presented dorsal colouration pattern changes (81 %, 13/16 individuals, Fig. 2 and Appendix 2); and some individuals belonging to the Giant orange morph underwent changes in dorsal colouration pattern (20 %, 2/10 individuals, Fig. 2 and Appendix 2). We do

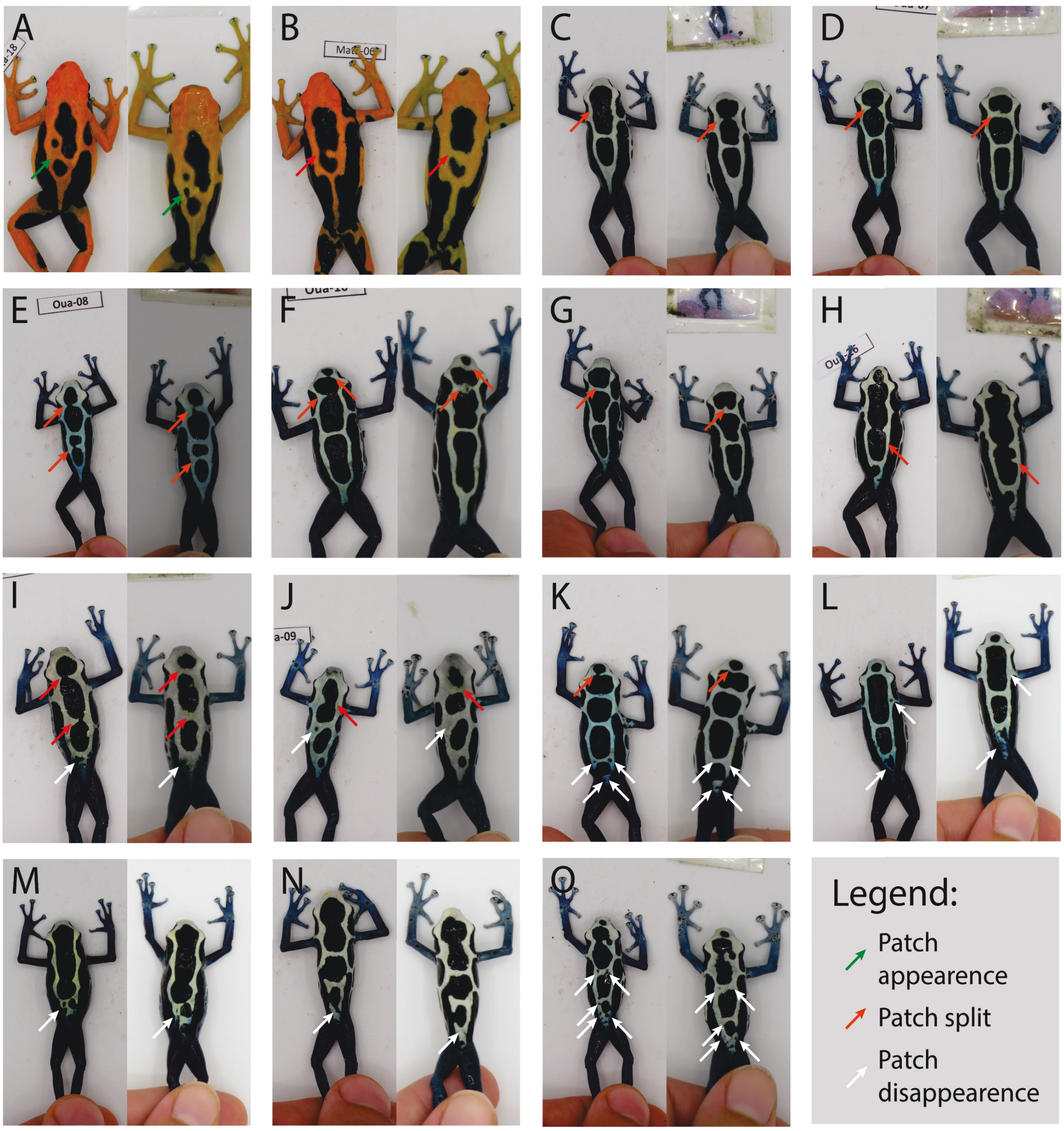

Figure 2. Dorsal colour pattern changes in *Dendrobates tinctorius* observed in 15 individuals within a 1–2-year period. Pictures are grouped by individual, presenting the dorsal colouration pattern at the time of capture (left) and after a 1–2-year elapsed period (right). Green arrows indicate patch appearances, red arrows indicate patch splits, and white arrows indicate patch disappearances. Individuals that did not undergo dorsal colouration pattern changes ($n = 21$) can be found in Appendix 2. Photographs of the first time point were taken by Ugo Lorioux-Chevalier, and photographs of the second time point were taken by Joana Sabino-Pinto.

not venture an explanation for the reason behind some colour-morphs being more stable than others.

Skin colouration patterns are a generalised trait used for individual identification in field studies, with the limitation that it should only be applied to adult individuals due to skin colouration pattern fixation (Schlüpmann and Kupfer, 2009). Here we show that, in one species in which this method has been applied, *Dendrobates tinctorius* (Born et al., 2010; Courtois et al., 2012; Rojas and Pašukonis, 2019), individuals undergo changes in dorsal colouration pattern throughout their life span. These changes can hamper long-term individual identification. We highlight that while these changes are often acknowledged in juveniles, our results show that they are also observed in fully developed adults (Fig. 2, Appendix 3). Furthermore, we present evidence that changes in dorsal colouration pattern might be correlated with colour-morph, with some morphs (Ouanary morph) being more prone to dorsal colouration pattern change than others (Nominal morph). Dorsal colouration pattern shifting, mostly in *D. tinctorius* juveniles, was initially described with the hypothesis that the area with aposematic colouration shrinks with age (Yuan et al., 2022), which is further supported by our data (Appendix 4). Here, we provide evidence that the colouration pattern changes observed throughout adulthood in wild individuals can also be quite drastic, which raises questions about how the function of colouration as an aposematic strategy varies at different times throughout an individual's life. In addition to the changes in dorsal colouration pattern mentioned above, we also observed melanisation following cicatrisation (snout of some of the Giant orange individuals, Fig. 2B and Appendix 2). This process has been observed in *Oophaga pumilio* and is known to lead to potential IIPR misidentifications (Meuche, 2009).

Both tested IIPR software programs showed very different outcomes when matching before and after pictures of the same individual. I3S proved to be the best program tested, being able to match the individuals correctly in the top 5 best matches 86.49 % of the time, and 97.30 % of the time in the top 20 best matches. Wild-ID was less successful, being able to provide a correct match in only 51.35 % and 75.68 % of the time in the top 5 and 20 best matches, respectively.

While some individuals maintain their dorsal colouration pattern over time, there is now enough evidence to exercise caution in limiting the identification of individual poison frog specimens based solely on dorsal colouration patterns, particularly for *Dendrobates tinctorius*. We thus warn researchers to be cautious about choosing IIPR software and for relying solely on dorsal colouration patterns (in this species) for individual identification in long-term studies. We recommend using a combination of dorsal/ventral/lateral colouration patterns.

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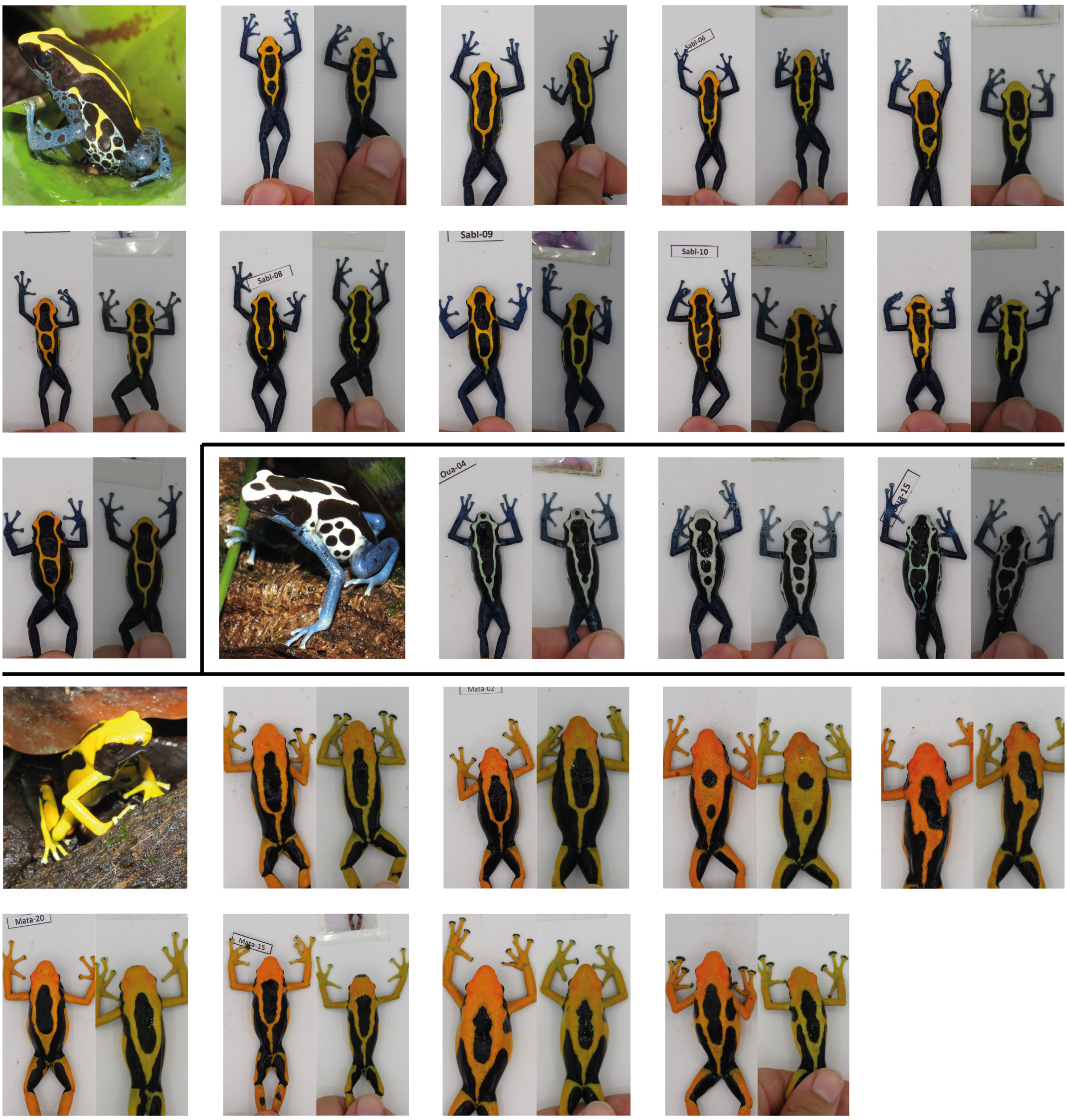
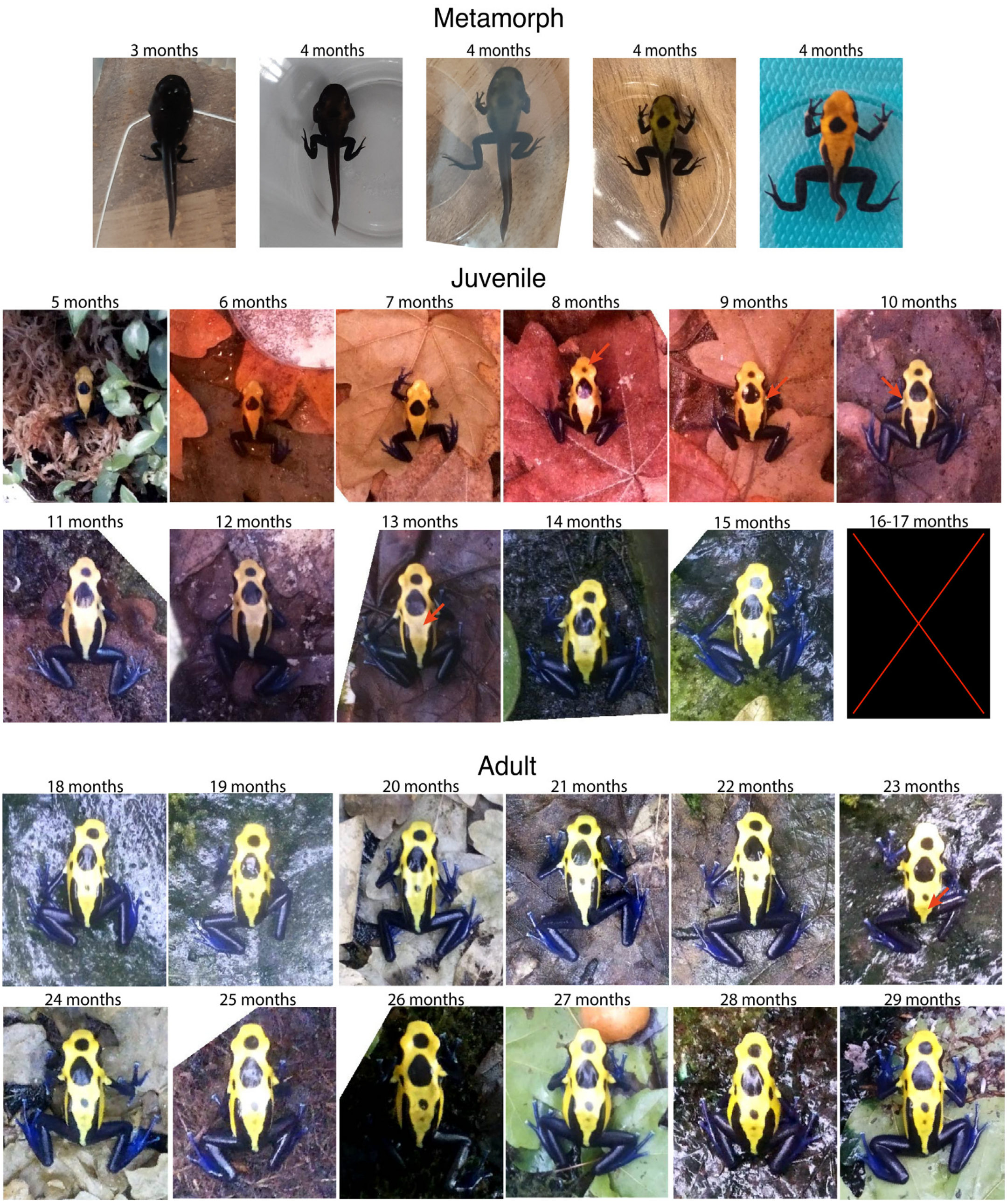
Appendix 1. Data table related to the experiment describing the time elapsed between pictures in months (Time between photos) and the sex (Sex) of each individual according to morph.

Individual	Time between photos	Sex
Nominal		
1	22	f
2	22	f
3	22	m
4	22	m
5	22	f
6	22	m
7	22	f
8	12	m
9	12	f
10	22	m
Ouanary		
1	22	f
2	22	f
3	22	m
4	22	f
5	22	m
6	22	f
7	22	m
8	22	m
9	22	m
10	22	m
11	22	m
12	22	f
13	22	m
14	22	f
15	22	f
16	22	f
Giant Orange		
1	12	m
2	12	f
3	12	m
4	12	f
5	12	f
6	12	f
7	12	f
8	12	f
9	8	m
10	8	m
11	8	f

Appendix 2. Wild caught individuals that did not undergo dorsal colouration pattern change throughout a 1–2 year period. Pictures are grouped by individual, presenting the dorsal colouration pattern at the time of capture (left) and after a 1–2-year elapsed period (right). Photographs of the first time point taken by Ugo Lorioux-Chevalier. Photographs of the second time point taken by Joana Sabino-Pinto.



Appendix 3. Dorsal colour pattern evolution in a captive-bred *Dendrobates tinctorius* individual from metamorphosis to adulthood. Adulthood was determined as in Yuan et al. (2022). Emerging spots are marked with a red arrow and can be seen at months 8–10, 13, and 23.



Appendix 4.

Study subject and housing. We tracked a captive bred individual for 2.5 years (2 months before metamorphosis, 18 months as juvenile, and 6 months as adult) to describe the type of pattern change during this period and to support previous literature.

A recently metamorphosed *D. tinctorius* (Cayenne morph) was placed in a glass terrarium (75x32x40 cm) with coconut soil, dried oak leaves, a water basin, live plants, and small plant pots as hiding places. The terrarium was kept on a 12–12 h light-dark cycle with an aquarium LED lamp (Nicrew). Terrarium temperature was controlled (25 ± 1 °C) with a heating pad (Exo Terra) through a custom Python program (https://github.com/marioasmira/frogfinder) running on a Raspberry Pi single-board computer. This individual was fed twice a week with flightless *Drosophila melanogaster* Meigen, 1830 dusted with a mix of Repashy® calcium plus as a nutritional supplement before feeding. The custom Python program also controlled a PiCamera that would record video for 1 min (altered to 30 sec, when the individual was ~2 years old) after movement was detected.

Image sampling and analysis. Each month, one frame of the videos was selected for dorsal colouration pattern analysis. Frame selection was based on whether the individual was close to the centre of the recording area and was in a horizontal position (which enables visualising most of the frog's dorsal surface).

Supplementary Results and Discussion

A *Dendrobates tinctorius* individual was followed from metamorphosis until adulthood in experiment 1 (Sup. Fig. 2). Due to technical issues, no picture is available for ages 16 and 17 months. During the juvenile phase, new black patches emerged at: month 8 in the head; month 9 at the right armpit; month 10 at the left armpit; and month 13 at the middle of the dorsum (back). During the adult phase, a new patch emerged in the lower back on month 23. We stopped tracking the dorsal colouration pattern at month 29. All black patches started as a slightly darker area in the yellow parts of the body that gradually became darker and larger. No patch merging was observed.

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