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# The importance of reproductive isolation in driving diversification and speciation within Peruvian mimetic poison frogs (Dendrobatidae)

Ugo Lorioux-Chevalier<sup>✉</sup>, Mathieu Chouteau & Alexandre-Benoit Roland<sup>✉</sup>

To explain how populations with distinct warning signals coexist in close parapatry, we experimentally assessed intrinsic mechanisms acting as reproductive barriers within three poison-frog species from the Peruvian Amazon belonging to a Müllerian mimetic ring (*Ranitomeya variabilis*, *Ranitomeya imitator* and *Ranitomeya fantastica*). We tested the role of prezygotic and postzygotic isolation barriers between phenotypically different ecotypes of each species, using no-choice mating experiments and offspring survival analysis. Our results show that prezygotic mating preference did not occur except for one specific ecotype of *R. imitator*, and that all three species were able to produce viable inter-population F1 hybrids. However, while *R. variabilis* and *R. imitator* hybrids were able to produce viable F2 generations, we found that for *R. fantastica*, every F1 hybrid males were sterile while females remained fertile. This unexpected result, echoing with Haldane's rule of speciation, validated phylogenetic studies which tentatively diagnose these populations of *R. fantastica* as two different species. Our work suggests that postzygotic genetic barriers likely participate in the extraordinary phenotypic diversity observed within Müllerian mimetic *Ranitomeya* populations, by maintaining species boundaries.

**Keywords** Adaptive diversification, Hybrid sterility, Mate choice, Prezygotic isolation, Postzygotic isolation

In the evolving landscape of speciation research, the past decades have marked a paradigm shift from geographic isolation to a more nuanced understanding of the mechanisms driving reproductive isolation and diversification amid gene flow<sup>1</sup>. Divergent selection is recognized as a crucial factor in promoting speciation, supported by both foundational and recent studies<sup>1–4</sup>. The accumulation of reproductive barriers such as behavioural or gametic isolation, hybrid sterility or unviability is pivotal in adaptive diversification, particularly in interconnected populations<sup>1,5–8</sup>. In this context, the study of prezygotic and postzygotic barriers could shed light on immediate biological processes and ultimate evolutionary trajectories.

Aposematic species, which advertise their unprofitability to potential predators, face strong natural selection favouring local uniformity, and leading to the counter-selection of migrants with exotic signals or hybrid phenotypes. The predation pressure on warning signals can thus create extrinsic prezygotic isolation between populations with distinct colour patterns, as observed in poison frogs<sup>9</sup> and butterflies<sup>10</sup>. This in turn can drive the evolution of intrinsic reproductive isolation mechanisms such as behavioural mate selection and genetic incompatibilities, limiting gene flow and recombination between individuals with distinct warning signals<sup>11</sup>. In the context of visually driven colour pattern diversification, mate choice leading to mating preference is hypothesized as a mechanism fostering prezygotic sexual selection<sup>12</sup>. Indeed, intermediate hybrid phenotypes risk higher mortality due to predator misidentification, favouring the evolution of such reinforcement mechanisms<sup>13</sup> toward colour pattern selection and divergence. On the other hand, intrinsic postzygotic barriers, such as genetic incompatibilities, changes in ploidy, structural genomic changes, meiosis defects owing to substantial DNA sequence divergence, or global patterns of inappropriate gene expression as a result of gene regulatory divergence can lead to hybrid sterility or reduced viability, contributing to reproductive isolation<sup>8,14–16</sup> and population divergence.

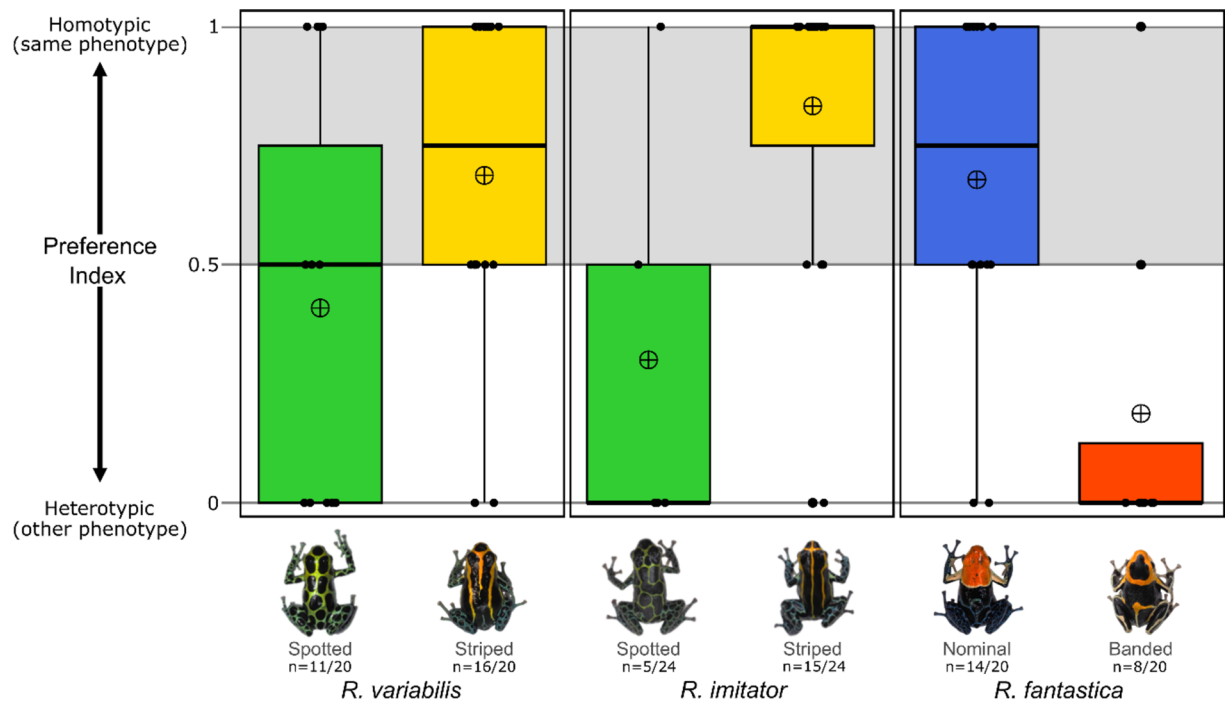
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The interplay of prezygotic and postzygotic mechanisms can lead to various scenarios of phenotypic diversification, potentially precipitating speciation events by preventing interbreeding. Understanding these proximate mechanisms is essential for deciphering the ultimate outcomes of species trajectories. Our study explores reproductive isolation mechanisms among three sympatric clades of aposematic poison frogs in Peru (*Ranitomeya fantastica*, *R. imitator* and *R. variabilis*), which are brightly coloured and chemically defended. For each species, their impressive diversity of warning signals is geographically structured between adjacent populations<sup>17–19</sup> revealing a geographic mosaic of aposematic signals with multiple ecotypes (2 for *R. variabilis*, 4 for *R. imitator* and up to 9 for *R. fantastica*). These species engage in Müllerian mimicry by converging locally into similar colour patterns, allowing to reduce the cost of associative learning to predators. In order for Müllerian mimicry to evolve effectively, aposematic models should have colonized areas prior to mimic species, and divergence time estimates indicate that the mimetic *R. imitator* indeed diverged more recently than its sympatric model species *R. variabilis* and *R. fantastica*<sup>19</sup>. Mimetic shift in *R. imitator* is associated with a narrow phenotypic transition zone, neutral genetic divergence and assortative mating, suggesting that divergent selection to resemble different model species has led to a breakdown in gene flow between some populations<sup>20</sup>. In addition, the highly diverse *R. fantastica* clade seems to have undergone recent speciation events with two species split from the rest of the clade (*R. benedicta* and *R. summersi*)<sup>21</sup>, and one ecotype (*R. fantastica* “white banded”) tentatively classified as *R. summersi*<sup>19</sup> (see Fig. 2 in Muell et al.<sup>19</sup>). The different levels of phenotypic diversification occurring between these three species (*R. imitator*, *R. variabilis* and *R. fantastica*) suggests that reproductive barriers might already be in place, but needed further investigations. Here we investigated if prezygotic barriers through sexual selection can impair the ability of divergent ecotypes to reproduce. Then we investigated the role of intrinsic postzygotic barrier in maintaining the amazing local adaptive diversity, by pedigree crossings and offspring survival among three mimetic *Ranitomeya* poison-frog species.

## Results

### Mate preference

To assess the role of prezygotic sexual selection, we conducted assays involving pairing similar and dissimilar frog ecotypes sequentially, and quantified realized mating events. For all three species tested in the 128 mating experiments, each individual experienced homotypic and heterotypic pairing in random order. Trial success (1) or failure (0) were scored and converted into an individual's preference index (Fig. 1). Of the three species and 6 ecotypes tested, only the striped *R. imitator* seems to have a significant preference for its own phenotype (Fig. 1 and Supplementary Table 1), while the others do not show any preference. These findings suggest that



**Fig. 1.** Prezygotic sexual selection: Individual phenotypic preference. For each animal paired in homotypic and heterotypic reproductive trials for 14 days, success (1) or failure (0) scores were converted into “preference index” (see “Methods”). Each dot corresponds to one frog and the boxplot presents the mean (cross dot), first and third quartiles, and whiskers extend to  $\pm 1.5 \times$  the interquartile range. Under each ecotype name is the number of individuals scored over the number tested after removing those that failed both trials. For *R. variabilis* and *R. imitator* (striped ecotype in yellow, spotted in green) for *R. fantastica* (nominal ecotype in blue, banded in red). ULC took the frogs pictures.

pre-mating barrier based on colour pattern selection seems surmountable and that frog's likely do not display mating preference except for one ecotype of *R. imitator*.

### Postzygotic barriers

To assess the role of postzygotic isolating barriers, specifically genetic incompatibilities, we conducted homotypic and heterotypic F0 pair crosses. We recorded development time and survival from hatching to metamorphosis. For development time, no significant differences between cross types were observed for *R. variabilis* and *R. fantastica*, but for *R. imitator*, heterotypic tadpoles developed faster (Supplementary Table 2). All F0 pairs produced fertilized clutches and viable F1 offspring (Fig. 2). To assess hybrid sterility, we conducted  $F1_{\text{hybrid}} \times F1_{\text{hybrid}}$  and  $F1_{\text{hybrid}} \times F0_{\text{parental}}$  crosses. Fertile clutches and viable offspring were produced for every hierarchical cross for *R. variabilis* and *R. imitator*, but not for *R. fantastica* (Fig. 2 and Supplementary Table 3). Notably, all crosses involving  $F1_{\text{hybrid}}$  *R. fantastica* males consistently produced clutches with a zero-survival rate (Fig. 2). These results indicate that postzygotic genetic incompatibilities act as a reproductive barrier between the two ecotypes of *R. fantastica*, likely representing distinct taxa. Additionally, the asymmetric sterility of hybrids aligns with Haldane's rule of speciation stating that: "When in the F1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex", suggesting that males could be the heterogametic sex.

### Discussion

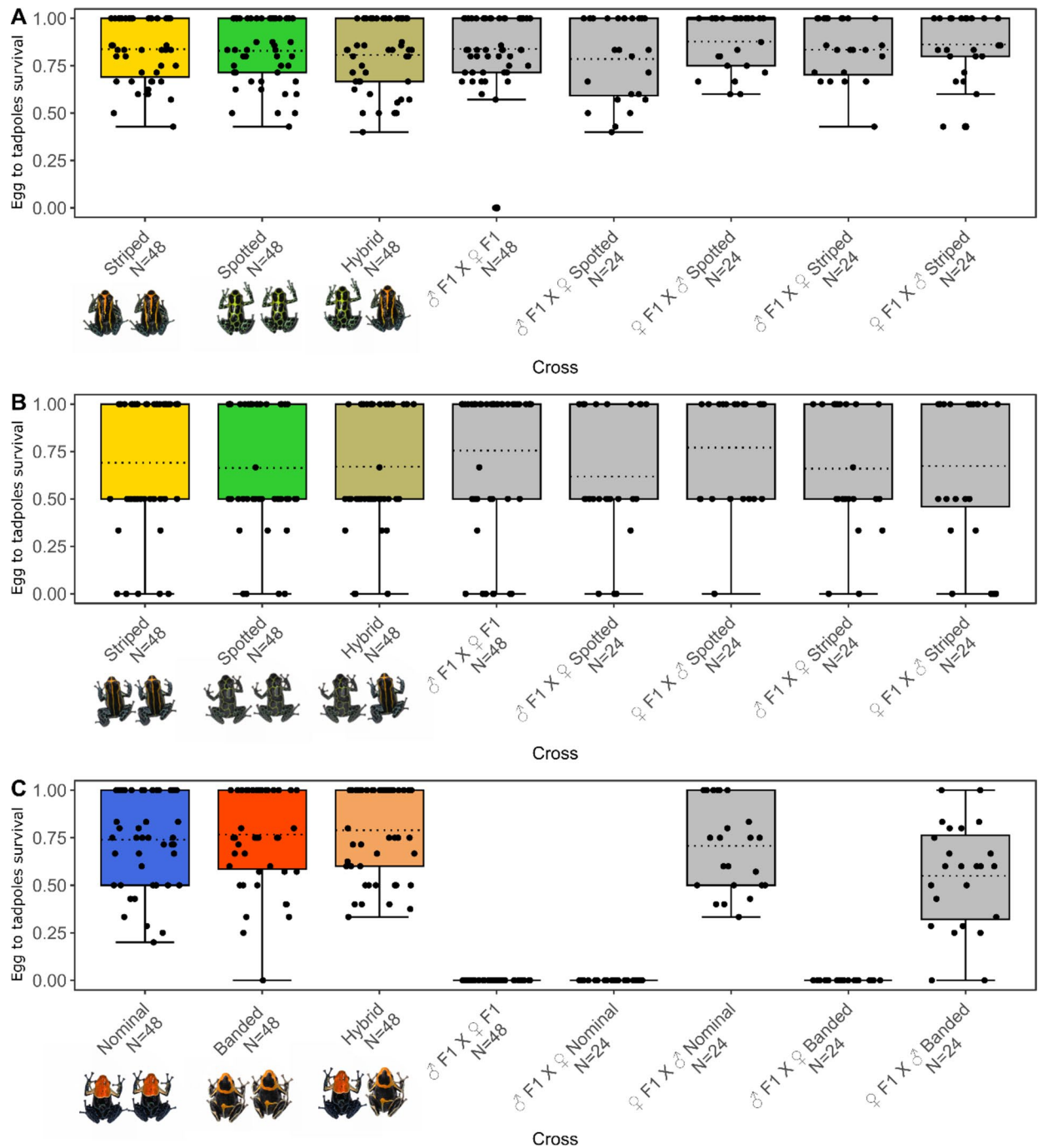
Predators plays an important role in the segregation and the maintenance of distinct ecotypes within aposematic species<sup>9</sup>, through positive frequency dependent selection. This mechanism eliminates exotic or intermediate ecotypes arising from migration or gene flow between different ecotypes, leaving only the most locally abundant form due to the fact that they have associated their ecotype with a secondary defence<sup>22</sup>. Nevertheless, the resulting selection can either be reinforced or counteracted by other prezygotic<sup>23</sup> and postzygotic barriers<sup>15</sup>. Our research delves into the early stages of reproductive isolation and genetic incompatibilities within the Peruvian *Ranitomeya* system, unravelling some proximate and ultimate mechanisms influencing speciation.

In anuran, vocalization or male calling play a critical role in mating behaviour and could be the first potential prezygotic barrier. Analysis of the advertisement calls could indicate if colour morphs are equally attractive to females, but based on literature we had no reason to think that the pairs of populations tested have high differences in advertisement calls<sup>17,21</sup>. In addition, a behavioural study on the highly variable dendrobatid *Oophaga pumilio* "found no evidence that females compared males by visiting them. Instead, females mated with the closest calling male irrespective of his acoustic and physical traits, and territory size"<sup>24</sup>.

Our study mainly focused on reproductive capacity between pairs of populations showing distinct phenotypes, and our primary interrogation was to unravel if divergent populations of the same species still have the capacity to interbreed and produce fertile offspring. Thus, we tested the role of prezygotic sexual selection using no-choice assay. While choice assay could have helped in deciphering if colour pattern is a criteria of mate selection, it is also biased and tends to overestimating preference strength. A no-choice experiment is actually equally sensitive to a choice assay when working on interspecies or interpopulation choice<sup>25</sup>. Our results show that there are no preferences toward similar individuals among *Ranitomeya* species, except for one specific ecotype of *R. imitator* in line with the results of another study<sup>20</sup>. Moreover, no variation of reproductive capacity between ecotypes was observed, all crosses being able to produce viable offspring. Taken together, behavioural or gametic barriers do not seem to strongly participate in prezygotic reproductive isolation, all three species being able to generate F1 generations that we used for testing intrinsic postzygotic barriers.

Our hierarchical pedigree crossings on the pairs of populations tested for *R. variabilis* and *R. imitator* were able to produce viable and fertile F1 up to F2 generations, showing that no genetic incompatibility exists to prevent these populations to freely interbreed. However, the unique pattern of F1 hybrid male sterility observed in *R. fantastica* suggests that the two populations belong to different species. One of the two ecotypes of *R. fantastica* in our study was recently categorized within *R. summersi* (our *R. fantastica* "banded" is *R. summersi* "white banded"<sup>19</sup>). The significant postzygotic reproductive isolation between our *R. fantastica* populations, consistent with Haldane's rule, strongly supports their status as distinct species, adding another brick to confirm the validity of this split<sup>17</sup>. According to this study, phenotypic divergence of ecotypes occurs in the same time frame for our three species: *R. fantastica*/*R. summersi* (1.78 Mya), *R. imitator* (0.91 Mya) and *R. variabilis* (1.85 Mya), but only *R. fantastica* shows genomic signs of speciation. This can likely be explained by genetic incompatibilities, emphasizing the crucial role of intrinsic reproductive barriers in triggering speciation.

Deciphering intrinsic postzygotic mechanisms in amphibians poses significant challenges, given that sex chromosome systems often deviate from the two famous rules of speciation: Haldane's rule and the large X-/Z-effect<sup>26</sup>. First, the widespread application of Haldane's rule encountered a breach in *Xenopus* frogs, where heterogametic ZW hybrid females, expected to be sterile, remained fertile<sup>27</sup>. In addition, no "sex" genes have been identified and sex-determination systems are still mostly unknown in Dendrobatoidea (including Dendrobatidae and Aromobatidae). So far, one species Aromobatidae (*Allobates femoralis*) and one in Dendrobatidae (*Adelphobates quinquevittatus*) seem to have heterotypic/heteromorphic sex chromosomes and both have a ZW sex-determination system. Although *R. fantastica* seems to verify Haldane rule, we have no evidence supporting that male could be the heterogametic (XY) sex. Second, the large X-/Z-effect, which suggests that heterogametic hybrids face genetic imbalance with an X or Z from only one parental species, is also challenged. This concept typically applies to well-differentiated (heteromorphic) sex chromosomes, as observed in mammals, birds, and certain insects. However, sex chromosomes have frequent recombination in amphibians and behave similarly to autosomes. This unique characteristic possibly leverages the ability of sex chromosomes to switch between male (XY) and female (ZW) heterogametic systems, maintaining a lesser degree of differentiation<sup>26</sup>. In Dendrobatoidea, 91% of the species tested (out of 21 species) seem to have homotypic/homomorphic sex chromosomes<sup>28</sup>.



**Fig. 2.** Postzygotic barriers: survival rate of egg to tadpole in F0 and F1 hybrid crosses for (A) *R. variabilis*, (B) *R. imitator*, (C) *R. fantastica*. Each dot corresponds to one clutch and N represents the number of egg clutches. The boxplot presents the mean in dotted-line, first and third quartiles and whiskers extend to  $\pm 1.5 \times$  the interquartile range. For *R. variabilis* and *R. imitator* (striped ecotype in yellow, spotted in green, hybrid in khaki) for *R. fantastica* (nominal ecotype in blue, banded in red and hybrid in orange), other F1 crosses are in grey. ULC took the frogs pictures.

But so far, without knowing sex determination system in *R. fantastica* we cannot rule out if it complies with Haldane rule or the large X-/Z-effect.

Several key genetic and epigenetic mechanisms can be attributed to 100% male sterility. Chromosomal incompatibility plays a major role, where mismatched chromosome sets from each parent fail to pair properly during meiosis, resulting in the production of non-viable sperm<sup>29,30</sup>. Additionally, epistatic interactions between genes from the different parental genomes as Bateson-Dobzhansky-Muller interaction can disrupt crucial processes in spermatogenesis, leading to a complete failure in sperm production<sup>31–33</sup>. Mitochondrial-nuclear incompatibility further compounds this issue, as disrupted interactions between nuclear and maternally inherited mitochondrial genes impair energy production necessary for sperm cell function<sup>34</sup>. Also, potential polyploidy, such as triploidy

in *Bufo* hybrid where sterile hybrid males have undeveloped or missing testes<sup>35</sup>, must be considered. However, hemiclinal reproduction in Dendrobatidae and *Ranitomeya* genus has never been documented, and triploidy is an extremely rare phenomenon, also it is difficult to conclude what type of genetic incompatibilities are more probably occurring in our study.

Because *R. fantastica* lack prezygotic mating and genetic barrier while showing strong intrinsic postzygotic barrier, genetic incompatibilities are likely maintaining its highly diverse mosaic of warning signals. Potent epistatic selective pressure against hybrid zygotes, coupled with higher predation on the hybrid/exotic warning colour pattern, likely contributes to maintaining locally adapted population boundaries by limiting gene flow. In the absence of both pre- and postzygotic reproductive isolation, likely happening in *R. variabilis* and partially in *R. imitator*, diversification of aposematic signals may occur through the interactions of genetic drift and spatial variation in the intensity of selection, facilitating local adaptation without a significant loss of fitness. The new phenotypes may then colonize alternative habitats if successfully recognized and avoided by predators, such mechanism participating in the process of phenotypic advergence proposed for Müllerian mimicry<sup>36</sup>.

## Conclusion

In summary, our research shows that prezygotic barriers through sexual selection are not influencing the maintenance of local adaptive diversity despite the fact that positive mating preference occurs at least in one ecotype of frog. Instead, a pattern of F1 hybrid sterility in one species, recalling Haldane's rule of speciation, suggests that intrinsic postzygotic incompatibilities, likely genetic epistatic interactions serve as a reproductive barrier, precipitating speciation and maintaining phenotypic diversity. These results underscore the importance of intrinsic mechanisms in speciation and showcases the diverse nature of reproductive barriers.

## Materials and methods

### Ethic statement

All experiments were carried out following the European directive for animal experimentation n°2010/63/UE, approved by the French Ministry of Higher Education and Research following the ethical submission APAFIS #38380-2022090708128291 and authorized by the Peruvian Servicio Nacional Forestal y De Fauna Silvestre -SERFOR (RDG N° D000442-2021-MIDAGRI-SERFOR-DGGSPFFS and 232-2016-17 SERFOR/DGGSPFFS) and were performed in accordance with relevant guidelines and regulations, no individuals were euthanized during the experiments. All the research members attended both the educational and training courses for the appropriate care and use of experimental animals approved by the French Ministry of Agriculture and Fisheries under the Directorate of Animal Health and Protection under N° I-13CNRS-F1-16. The experimental protocols were conducted in accordance with the ARRIVE guidelines.

### Model species

*Ranitomeya variabilis*, *R. imitator* and *R. fantastica* are aposematic dendrobatid frogs found in sympatry in the states of San Martín and Loreto in northern Peru (Supplementary Fig. 1). Their colour patterns, associated with chemical defences, are locally influenced by predator pressures creating a mosaic of warning signals along their distribution range. Despite being subject to the same selective forces, these three species have a different number of ecotypes: 2 for *R. variabilis*, 4 for *R. imitator* and up to 9 for *R. fantastica*<sup>17</sup>. Interestingly, due to the selection pressure exerted by predators, they converge phenotypically in some locality into a Müllerian mimicry ring.

*R. variabilis* usually lay eggs in bromeliads, *R. imitator* in *Heliconia* and *Xanthosoma*, while *R. fantastica* mostly lay them on the forest floor. After hatching, they all transport their cannibalistic tadpoles into separate phytotelmata. The three species provide different levels of parental care, from male egg guarding and tadpole transport in *R. variabilis* and *R. fantastica*, to biparental care with tadpole's egg-feeding in *R. imitator*.

### Sample collection

Between 2015 and 2022, we gathered a total of 236 wild individuals from three *Ranitomeya* frog species at five locations in Peru (refer to Suppl. Figure 1 for the localities and names): *R. variabilis* (76 individuals, 2 ecotypes: San-Jose ("Spotted") and Varadero ("Striped")), *R. imitator* (84 individuals, 2 ecotypes: San-Jose ("Spotted") and Varadero Banda ("Striped")), and *R. fantastica* (76 individuals, 2 ecotypes: Micaela ("Nominal") and Bocatoma ("Banded")). Each individual was photographed in the lab with an Olympus OM-D EM-5 camera coupled with a M.Zuiko 60 mm macro lens, and assigned a unique ID for identification and tracking within the colony.

All data analyzed during this study are included in this published article in supplementary.

### Captive collection and raising conditions

All *Ranitomeya* breeding and crossings were conducted at the Tarapoto Research Center (San Martín, Peru), situated within their natural distribution range. The frogs were housed either in breeding pairs or groups of ten for juveniles, accommodated in 60 × 30 × 30 cm glass terrariums equipped with water drainage, a natural hardscape, and live plants for enrichment. These terrariums were placed in a mesh greenhouse with natural ventilation and a misting system to replicate their native environment. The frogs were fed wingless fruit flies (*Drosophila melanogaster*) and springtails at least three times a week, supplemented with a mix of calcium and vitamins once a week (*Repashy® Supercal NoD* and *Supervite*). Within the breeding enclosures, artificial sites tailored to species-specific needs for oviposition were provided. For instance, *R. fantastica*, which uses covered leaf litter as a deposition site, had 15 cm pipe sections placed horizontally on the ground. *R. imitator*, which uses hollow treeholes or large petioles, had 20 cm pipe sections placed at a 60° angle from the ground to mimic their phytotelmata (*Xanthosoma*, *Heliconia*, etc.). *R. variabilis*, which uses Bromeliad phytotelmata, had 5 cm pipe sections sealed on one side placed on the terrarium wall and tilted at a 10° angle, 15 to 20 cm above the ground.

### Prezygotic sexual selection

In *Ranitomeya*, males typically call from exposed perches to attract females during courtship. Realized mating, which reflects the various aspects of mate choice by both sexes leading to a successful mating event, was employed to quantify sexual selection.

We used a straightforward no-choice behavioural assay, mimicking a realistic encountering interaction between a migrant individual with exotic warning signal and a potential mate with the native colour-pattern. 60 × 30 × 30 cm glass terrariums equipped with water drainage, a natural hardscape, and live plants for enrichment were used, female were placed in each terrarium first then males. We recorded the time to the first egg clutch for each pair of frogs over a trial period of 14 days, the males were then interchanged in order that each individual pass into homotypic and heterotypic pairs. We conducted a series of homotypic and heterotypic pair crosses for each the three focal species as follows: homotypic ( $\sigma_{\text{morph-A}} \times \varphi_{\text{morph-A}}$ , n = 10–12 biological replicates (pairs) and  $\sigma_{\text{morph-B}} \times \varphi_{\text{morph-B}}$ , n = 10–12 biological replicates) and heterotypic ( $\sigma_{\text{morph-A}} \times \varphi_{\text{morph-B}}$ , n = 10–12 biological replicates and  $\sigma_{\text{morph-B}} \times \varphi_{\text{morph-A}}$ , n = 10–12 biological replicates). For each animal paired in homotypic and heterotypic reproductive trials for 14 days, success (1) or failure (0) scores were converted into “preference index” which was calculated as follow:

$$\text{Preference Index} = \frac{\text{Homotypic Score}}{\text{Homotypic Score} + \text{Heterotypic Score}}$$

Individuals that failed in both trials were removed to avoid division by zero. Values range from 0 to 1 meaning: 1 being a preference for similar phenotype (positive mating preference), 0 a preference for dissimilar phenotype (negative mating preference) and a value of 0.5 shows no preferences. Data normality was assessed using the Shapiro test, and we used the nonparametric Wilcoxon signed rank test to compare the median of each ecotype against the hypothetical median (0; 0.5; 1).

### Postzygotic genetic incompatibilities

To evaluate and quantify the impact of intrinsic postzygotic genetic incompatibility on adaptive diversity, we conducted hierarchical crosses for each of the three species. A total of 3513 eggs were followed (1626 for *R. variabilis*, 510 for *R. imitator* and 1377 for *R. fantastica*), and compared between treatments.

Initially, we generated three parental crosses (F0): homotypic ( $\sigma_{\text{morph-A}} \times \varphi_{\text{morph-A}}$ , n = 6 biological replicates;  $\varphi_{\text{morph-B}} \times \sigma_{\text{morph-B}}$ , n = 6 biological replicates) and heterotypic (F0 Hybrids):  $\sigma_{\text{morph-A}} \times \varphi_{\text{morph-B}}$  (n = 3 biological replicates);  $\sigma_{\text{morph-B}} \times \varphi_{\text{morph-A}}$  (n = 3 biological replicates) for the three species. We measured the survival rate (equation below) and development time for each of these crosses, to determine whether heterotypic and homotypic crosses are different (Total clutch number n = 432: *R. variabilis* n = 144, *R. imitator* n = 144, and *R. fantastica* n = 144. Total egg number n = 1766: *R. variabilis* n = 831, *R. imitator* n = 265, and *R. fantastica* n = 670).

$$\text{survival rate(per clutch)} = \frac{\text{number of hatched larva}}{\text{number of laid eggs}}$$

Then we followed a stepwise process for each of the three species, to assess if major genetic incompatibility results in F1 morph-hybrid adult sterility. Non-related  $\sigma_{\text{F1-hybrid}} \times \varphi_{\text{F1-hybrid}}$  (n = 6 biological replicates) crosses were conducted to determine egg development and mortality. To ascertain whether sterility is sex-dependent, we proceeded with  $\sigma_{\text{F1-hybrid}} \times \varphi_{\text{F0-morph-A}}$  (n = 3 biological replicates);  $\sigma_{\text{F0-morph-A}} \times \varphi_{\text{F1-hybrid}}$  (n = 3 biological replicates);  $\sigma_{\text{F1-hybrid}} \times \varphi_{\text{F0-morph-B}}$  (n = 3 biological replicates);  $\sigma_{\text{F0-morph-B}} \times \varphi_{\text{F1-hybrid}}$  (n = 3 biological replicates). We measured the survival rate (equation above) and development time for each of these crosses (Total clutch number n = 432: *R. variabilis* n = 144, *R. imitator* n = 144, and *R. fantastica* n = 144. Total egg number n = 1747: *R. variabilis* n = 795, *R. imitator* n = 245, and *R. fantastica* n = 707).

Data normality was assessed using the Shapiro test, followed by a Kruskal–Wallis and pairwise Wilcoxon test to analyse differences in egg-to-tadpole survival rates between crosses.

### Data availability

Raw data is provided on the supplementary materials under the following names: DVP\_time.xlsx: Contains columns for species, cross type, and number of days from froglet to tadpole. data\_postzyg.xlsx: Includes columns for species, cross type, and survival rate from egg to tadpole. data\_prezyg.xlsx: Features columns for species, cross type, male ID, female ID, cross ID, number of days before the first egg laying, male population type, female population type, number of eggs laid, treatment, and a binary reproduction classification.

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## Author contributions

M.C. conceptualized the study and acquired funding; U.L.C. and M.C. collected data; U.L.C. and A.B.R. analysed data and prepared the original draft; all authors reviewed and revised the manuscript; U.L.C. took the frogs pictures.

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## Competing interests

The authors declare no competing interests.



### Additional information

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