Advergence in Müllerian mimicry: the case of the poison dart frogs of Northern Peru revisited

Mathieu Chouteau1*, Kyle Summers2, Victor Morales3 and Bernard Angers1

1Department of Biological Sciences, Université de Montréal, C.P. 6128, Succursale Centre Ville, Montreal, Canada, H3C 3J7
2Department of Biology, East Carolina University, Greenville, NC 27858, USA
3Universidad Ricardo Palma, Apartado Postal 18-01, Benavides 5440, Surco, Lima 33, Peru
*Author for correspondence (mathieu.chouteau@umontreal.ca).

2. MATERIAL AND METHODS

The sampling was performed in the department of San Martín (Peru) in two adjacent localities separated by ca 16 km (figure 1). A minimum of 15 individuals per species were caught in these localities (table 1).

Received 1 January 2011 Accepted 21 February 2011

Population genetics

The diversification of aposematic signals in Müllerian mimicry is a puzzling phenomenon, because mimicry is expected to promote uniformity in warning signals [1]. Yet substantial variation in aposematic signals has been shown in, for example, Neotropical butterflies [2] and poison dart frogs [3] throughout their range. Such diversification raises the question of whether both sympatric species change towards a common phenotype (i.e. convergence) or if the phenotype of one species moves towards the phenotype of another species that remains constant (i.e. advergence) as expected in Batesian mimicry [4]. Advergence is considered more likely when one species’ aposematic signal is established before the arrival of a second species [3,5]. Empirical evidence, principally available indirectly for the putative case of Ranitomeya imitator, has thus far tended to support advergence [6,7].

Symula et al. [3] concluded, using a small number of individuals per population, that the phenotypically variable R. imitator mimics distinct model species in different geographical areas. This was supported by the small divergence measured between mitochondrial haplotypes of different R. imitator colour morphs compared with that observed between model species, suggesting that R. imitator had undergone a relatively recent geographical radiation during which it adopted the aposematic signals of the distinct model species it encountered [3].

However, owing to the importance of this textbook example in the field of evolutionary biology of mimicry [6,7], we re-addressed the theory of advergence in the R. imitator system using population genetics, in the localities originally used for the description of the mimicry association [3]. We compared two adjacent but phenotypically distinct populations of R. imitator and its local sympatric model species, Ranitomeya ventrimaculata and Ranitomeya variabilis. We specifically tested the following predictions in support of advergence: (i) the model species display higher genetic differentiation when compared with R. imitator populations, and (ii) R. imitator is more phenotypically variable within and between regions than the model species [5].
following qualitative bins based on their hue: light orange (30°–60°), yellow (61°–90°) and green (91°–120°). The dorsal pattern in the localities studied consisted of three well-separated longitudinal stripes with different degrees of reticulation between them. Each individual was assigned to a qualitative bin based on the amount of reticulation: no reticulation (0), slightly reticulated (1–2), reticulated (3–4) and highly reticulated (5+). Comparison of variance between sites was assessed using Levene’s test (W).

3. RESULTS

Both the mitochondrial and nuclear markers displayed high diversity within populations (table 1). The mitochondrial minimum spanning network (figure 2a) revealed that populations of *R. imitator* are characterized by distinct haplotypes, with a mean number of 1.84 mutations between populations. On the other hand, the haplotypes of *R. ventrimaculata*/*variabilis* are intermingled and both species had one common haplotype in high frequency, resulting in a number of mutations (0.14) one order of magnitude lower than that of *R. imitator*. All populations were in Hardy–Weinberg equilibrium for nuclear markers and exact tests of differentiation are significant (p < 0.001). The estimates of

---

**Table 1. Genetic variability and differentiation estimated from nuclear and mitochondrial markers between populations of *Ranitomeya imitator* and the model species. (The associated probabilities after 999 random permutations are indicated in parenthesis. Test for relevance of molecular information is included for both the nuclear loci and mitochondrial control region.)**

<table>
<thead>
<tr>
<th></th>
<th><em>R. imitator</em></th>
<th><em>R. ventrimaculata</em></th>
<th><em>R. variabilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lowland</td>
<td>highland</td>
<td>lowland</td>
</tr>
<tr>
<td>lowland</td>
<td>n = 20</td>
<td>n = 22</td>
<td>n = 15</td>
</tr>
<tr>
<td>highland</td>
<td>n = 22</td>
<td></td>
<td>n = 16</td>
</tr>
<tr>
<td>number of haplotypes</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>probability that $\varphi_{ST} &gt; F_{ST}$</td>
<td>p = 0.042</td>
<td></td>
<td>p = 0.523</td>
</tr>
<tr>
<td>number of alleles per loci (mean ± s.d.)</td>
<td>11.2 ± 2.1</td>
<td>11 ± 3.4</td>
<td>12.5 ± 3.3</td>
</tr>
<tr>
<td>$H_T$ (mean ± s.d.)</td>
<td>0.863 ± 0.065</td>
<td>0.861 ± 0.038</td>
<td>0.879 ± 0.044</td>
</tr>
<tr>
<td>$F_{ST}$ (95% CI)</td>
<td>0.103 (0.092–0.118)</td>
<td>(p &lt; 0.001)</td>
<td>0.023 (0–0.041) (p = 0.034)</td>
</tr>
<tr>
<td>$R_{ST}$ (95% CI)</td>
<td>0.074 (0.051–0.098) (p &lt; 0.001)</td>
<td>0.206 (0.123–0.266) (p &lt; 0.001)</td>
<td>0.128 (0.098–0.157) (p &lt; 0.001)</td>
</tr>
<tr>
<td>$\varphi_{ST}$ (95% CI)</td>
<td>0.901 ± 0.076</td>
<td>0.432 ± 0.132</td>
<td>0.670 ± 0.081</td>
</tr>
<tr>
<td>$F_{ST}$</td>
<td>0.343 (p &lt; 0.001)</td>
<td>0.083 (p = 0.069)</td>
<td>0.115 (p = 0.019)</td>
</tr>
<tr>
<td>probability that $\varphi_{ST} &gt; F_{ST}$</td>
<td>p = 0.596 (p &lt; 0.001)</td>
<td>p = 0.438</td>
<td></td>
</tr>
</tbody>
</table>

*Differentiation measured using the four loci displaying the lowest differentiation.
differentiation assessed with mtDNA and nDNA (table 1) are higher between populations of *R. imitator* than between the model species, even when the four loci displaying the lowest differentiation are used to compensate for the different number of loci available between species. SPAGeDI results confirm that the use of the mutational information (*F*<sub>ST</sub> versus *R*<sub>ST</sub>) is relevant for *R. imitator* but not between model species (table 1). This is concordant with the STRUCTURE results, where *R. imitator* individuals are clearly separated into two populations according to their geographical location, while individuals of *R. ventrimaculata/variabilis* failed to be properly assigned to their species of origin for all *K*-values tested (figure 2b).

The comparison of phenotypic variability between *R. ventrimaculata* and *R. imitator* living in the lowland localities (\(W_{1,6} = 0.194, p = 0.675\)) and between *R. variabilis* and *R. imitator* in the highland (\(W_{1,6} = 0.200, p = 0.625\)).
0.286, \( p = 0.612 \) show that both species display similar levels of variation and that they share the same aposematic signal in each locality (figure 2c). When the results for both localities are pooled, \( R. \) imitator and \( R. \) ventrimaculata/\( variabilis \) exhibit the same level of phenotypic variability (\( W_{1,4} = 0.007, p = 0.936 \)).

4. DISCUSSION
The predictions of the advergence hypothesis in \( R. \) imitator, regarding genetic differentiation and phenotypic variability [3,5], were not supported by the results of the present study.

Results of this study revealed that, in spite of a clearly distinct aposematic signal [15], \( R. \) ventrimaculata and \( R. \) variabilis display very low genetic differentiation. Incomplete sorting of mtDNA haplotypes, \( F_{ST} \) values not significantly different from zero and the inability to assign individuals to its species of origin (\textsc{structure}) clearly indicate a recent separation of these groups. While it is not possible in the absence of data on reproductive isolation to determine whether \( R. \) ventrimaculata and \( R. \) variabilis are populations of the same species or distinct species, we can conclude that these two groups were recently connected by a common ancestor in both of these scenarios. This is consistent with previous phylogenetic analyses which show that \( R. \) ventrimaculata is a polyphyletic taxa characterized by the \( R. \) variabilis and \( R. \) ventrimaculata from our studied localities being closely related but clearly different from other \( R. \) ventrimaculata [16].

These findings are important because the identification of the mimicking species is in part based on phenotypic variability; i.e. the mimicking species is expected to be more phenotypically variable across localities than its model species [5]. Since \( R. \) ventrimaculata and \( R. \) variabilis belong to the same lineage and have most probably diverged recently, they are in fact as variable as \( R. \) imitator in the present system.

Another striking result is that the populations of \( R. \) imitator are far more genetically differentiated than those of \( R. \) ventrimaculata/\( variabilis \) for both genomes. This result is inconsistent with the results of Symula et al. [3], whose objective was to assess the Müllerian mimicry relationship but not its direction. The difference is most probably the result of the small number of individuals analysed by Symula et al. [3], which prevented them from accurately assessing the levels of differentiation between these genetically diverse species. This casts doubt on the hypothesis that populations of \( R. \) imitator diversified after the model species and adopted their aposematic signal [3]. To assess the directionality in mimicry, the chronology of founding events in a given locality is essential. However, it is also important to note that genetic differentiation may represent an inappropriate measure of this chronology in the absence of complete phylogeographic information.

Symula et al. [3] also discussed a third species, \( Ranitomeya \) summersi (previously called \( Ranitomeya \) fantastica), which is mimicked by another colour morph of \( R. \) imitator. Because this clade is genetically very distinct from both \( R. \) imitator and \( R. \) ventrimaculata/\( variabilis \), it could be argued that genetic differences between the model species remain higher than among the \( R. \) imitator colour morphs. However, numerous other colour variants in the \( R. \) fantas- ticus clade have been discovered since the initial description of the system, some of which are sympatric and involved in mimicry with both \( R. \) imitator and \( R. \) ventrimaculata/\( variabilis \) [17]. Because all species involved in this Müllerian mimicry show high phenotypic variability, conclusions from phenotypic variability or genetic distances between clades remain inconclusive in this system.

In conclusion, our results cast doubt on the evidence previously used to infer the hypothesis of mimetic advergence in the \( Ranitomeya \) species, as our results contradict two key predictions used in the initial description of the system. This study is of particular importance because the \( R. \) imitator system is commonly considered to provide the strongest empirical evidence for advergence [6,7]. This does not mean that the theory of advergence is false or that \( R. \) imitator is not the mimic, but rather that no empirical evidence exists as of yet. This study reopens the discussion concerning the direction of mimicry in the \( R. \) imitator system.

The experiments were approved by the University of Montreal’s ethics committee and by the Instituto Nacional de Recursos Naturales del Peru which provided the collecting and export permits: no. 005-2008-INRENA-IFFS-DCB and CITES no. 11067.

The authors would like to thank Melanie McClure, Méloé Prud’Homme, Christelle Leung and Jason Brown for their valuable help. This research was supported by a Natural Sciences and Engineering Research Council scholarship of Canada to M.C.

1 Müller, F. 1878 Über die Vortheile der Mimicry bei Schmetterlingen. \textit{Zoologischer Anzeiger} 1, 54–55.
10 Brown, J. L., Chouteau, M., Glenn, T. & Summers, K. 2009 The development and analysis of twenty-one microsatellite loci for three species of Amazonian...


