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Molecular phylogeny of Malagasy poison frogs, genus *Mantella* (Anura: Mantellidae): homoplastic evolution of colour pattern in aposematic amphibians

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Abstract

We studied the evolution of colour pattern in Malagasy poison frogs, genus *Mantella*, a group of diurnal and toxic frogs endemic to Madagascar. Based on a phylogeny reconstructed using 1130 bp of the mitochondrial 16S rRNA gene, the genus can be divided into five species groups. Within some of these groups, interspecific genetic divergences were very low (1.2–2.8% sequence divergence) while colour patterns were markedly different. In contrast, *Mantella madagascariensis* and *M. baroni*, two species which show extremely similar dorsal coloration patterns, were not included in the same clade. This conclusion was supported by high bootstrap values and by significant rejection of alternative topologies using KH-tests. Analysis of colour patterns and tentative reconstruction of ancestral states yielded five character states shared by these two species but not by their respective sister species, *M. aurantiaca* and *M. nigricans*. Considering these detailed similarities as symplesiomorphic therefore requires the assumption of multiple reversals in other species, whereas a homoplastic colour evolution in the sympatric *M. madagascariensis* and *M. baroni* appears as most parsimonious. This parallelism may have been triggered by Müllerian mimicry. However, additional data is necessary to support this hypothesis.

Key words: Amphibia, Mantellidae, Madagascar, 16S rRNA, phylogeny, aposematism, mimicry

See also Electronic Supplement at **http://www.senckenberg.de/odes/02-04.htm**

Introduction

Eye-catching colour and pattern is widespread among animals, and may serve different functions in intra- and interspecific signalling, especially in courtship and sexual display. Individual differences in colour can be indicative of fitness to partners or rivals. For example, Hamilton & Zuk (1982) predicted a positive association between brightness and parasite prevalence. Conspicuous coloration in toxic or unpalatable organisms is referred to as warning coloration or aposematism, and it seems clear that its function is to deter predation (Servedio 2000).

Frogs display a wide array of colour and pattern, but most are rather cryptic and only few groups can be characterised as genuinely aposematic. This is comprehensible because most anurans are nocturnal, thus inter- or intraspecific recognition of exceptional coloration is unlikely. Nevertheless, a number of anurans are prominent for their vibrant colours and patterns. These vividly coloured species are mostly diurnal, and toxic. The best known example is the South American family Dendrobatidae (dart poison frogs): species of the genera *Dendrobates* and especially *Phyllobates* accumulate highly toxic alkaloids in their skin (Daly et al. 1987), and their toxicity appears to correlate with aposematic coloration (Summers & Clough 2001).

Dendrobatids obtain their alkaloid poisons through uptake from prey (Daly et al. 1994), and many species are specialised ant eaters (Caldwell 1996). A very similar case is found in the unrelated Malagasy poison frogs, genus *Mantella* Boulenger, 1882 (family Mantellidae;

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Vences & Glaw 2001). These frogs also are microphagous ant-eaters (Vences et al. 1998a), and in all species studied so far the alkaloid composition of their skin is similar to that of dendrobatids (Daly et al. 1996).

Mantella are small, largely diurnal, and often colourful frogs that are endemic to Madagascar (Vences et al. 1999) (see colour plates in Part 1 of the Organisms Diversity and Evolution Electronic Supplement 02–04, available at http://www.senckenberg.de/odes/). Most species live in the eastern rainforests while a few can also be found in coastal lowland areas, often outside forested areas. Vences et al. (1999) recognised 17 species, which they divided in six species groups. In the past the number of valid *Mantella* species and the taxonomy of the genus have been controversial issues (e.g., Daly et al. 1996). Until recently, species descriptions have been largely based on colour pattern (e.g., Pintak & Böhme 1988, 1990; Busse & Böhme 1992). As a result, *Mantella madagascariensis* and *M. baroni*, two forms with a highly similar colour pattern, have only recently been recognised as genetically well differentiated species (Vences et al. 1998c). Indeed, based on their allozyme and osteological data Vences et al. (1999) placed *M. madagascariensis* (Grandidier, 1872) and *M. baroni* Boulenger, 1888 into two different species groups. Glaw & Vences (2000) concluded that these two taxa could represent an example for Müllerian mimicry among amphibians, an hypothesis already advanced by Andreone (1992) for the superficially similar *M. pulchra* Parker, 1925 and *M. baroni*.

Although bioacoustic, karyological, morphological, osteological and genetic data on *Mantella* are available (Pintak et al. 1998; Vences et al. 1998b, c, 1999), a conclusive intrageneric phylogeny has not yet been published. Our objectives in the present study were thus twofold. Firstly, we wished to derive a phylogeny of *Mantella* using mitochondrial 16S rRNA sequence data. Secondly, we examined the evolution of dorsal patterns in *Mantella* using our molecular phylogenetic hypothesis.

Material and methods

Specimens examined

We sequenced two fragments of the mitochondrial 16S rRNA gene of 23 specimens of 14 *Mantella* species, as well as of three outgroup taxa (see Appendix for taxa, voucher specimens and GenBank accession numbers). The species represent the six *Mantella* species-groups as described by Vences et al. (1999): *Mantella laevigata* (*M. laevigata* group); *M. betsileo, M. expectata, M. viridis* (*M. betsileo* group); *M. aurantiaca, M. crocea, M. milotympanum* (*M. aurantiaca* group); *M. bernhardi* (*M. bernhardi* group); *M. baroni, M. cowani, M. haraldmeieri, M. nigricans* (*M. cowani* group); *M. madagascariensis, M. pulchra* (*M. madagascariensis* group).

Choice of outgroup

As the major outgroup we used *Mantidactylus grandisonae* Guibé, 1974, a representative of the subgenus *Blommersia* which previously had been determined to be the sister taxon of *Mantella* (16S and 12S rRNA sequence analysis of representatives of *Mantella* and of all but one subgenus of *Mantidactylus;* H.-C. Schaefer, unpublished data). For hierarchical outgroup rooting we included *Mantidactylus grandisonae*, *Mantidactylus bicalcaratus* (Boettger, 1913), and *Boophis tephraeomystax* (Duméril, 1853) (subfamily *Rhacophorinae*).

Amplification and sequencing

DNA was extracted from fresh or ethanol (70%) preserved muscle tissue using a Boehringer Mannheim extraction kit following the manufacturers protocol. Two sections of the mitochondrial 16S rRNA gene were amplified using polymerase chain reaction (PCR). We used the primers 16SA-L and 16SB-H, modified from Palumbi et al. (1991) and Kocher et al. (1989). For the amplification of the second portion of the 16S rRNA gene we used the primers 16SA-H (reverse of 16SA-L) and 16L3 (modified from Hedges 1994): AGC AAA GAH YWW ACC TCG TAC CTT TTG CAT. For all primer sequences and cycling protocols, see Vences et al. (2000) and Mausfeld et al. (2000). PCR was performed using Amersham Pharmacia Biotech Ready To Go® PCR beads. The Boehringer Mannheim PCR Product Purification Kit was used to purify the PCR products. Cycle sequencing and sequence determination was done with ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Mix and an ABI PRISM 377 automatic sequencer.

Sequence analysis

Sequences were provisionally aligned with ClustalX (Thompson et al. 1997) and subsequently adjusted manually (the alignment is available from Electr. Suppl. 02–04, Pt 2). Phylogenetic analyses were peformed with PAUP*, version 4.0b8 (Swofford 2001). Maximum parsimony (MP) trees were calculated in two ways: with gaps coded as fifth base or as missing characters. The bootstrap method was performed with heuristic searches. The following options were selected: starting tree(s) was obtained via stepwise addition in input order. One tree was held at each step during stepwise addition. For branch-swapping we used the tree-bisection-reconnection (TBR) algorithm. The steepest descent option was not in effect, while the "MulTrees" option was in effect. Topological constraints were not enforced. The Neighbor Joining (NJ) tree was inferred using the logDet method, which calculates the determinants of pairwise 4×4 nucleotide substitution matrices and is robust against possible variation of sequence evolution among lineages (Lockhart et al. 1994).

In order to explore which substitution model fits our sequence data best for the Maximum Likelihood analysis, we applied a hierarchical likelihood ratio test for testing the goodness-of-fit of nested substitution models using the program MODELTEST version 3.04 (Posada & Crandall 1998) prior to phylogenetic reconstruction. The parameters estimated by MODELTEST were then used to obtain a Maximum Likelihood (ML) tree, using the heuristic search option.

Following Hedges (1992) we ran 2000 bootstrap replicates (Felsenstein 1985) in all analyses except ML, where only 100

replicates were run because of computational constraints. Only bootstrap values ≥70% indicate sufficiently resolved topologies (Huelsenbeck & Hillis 1993), those between 50% and 70% were regarded as tendencies. Additionally, it was tested whether alternative trees fit the sequence data significantly worse than the best tree using Kishino-Hasegawa likelihood ratio tests (Kishino & Hasegawa 1989) as implemented in PAUP* (RELL bootstrap, 1000 replicates, one-tailed test - see below for all tested topologies).

Results

The combination of the two fragments of the 16S rRNA gene yielded a data matrix of 1130 bp. Of these, 761 bp were constant, 133 bp were variable but parsimony-uninformative, and 236 bp were parsimony-informative. MODELTEST estimated a gamma shape parameter of 0.3676 for variable sites and a proportion of invariable sites of 0.3709. Base frequencies were A = 0.2797, C = 0.1671, G = 0.1907, T = 0.3625. Substitution rates were: [A-C] = 1.0000, [A-G] = 8.9868, [A-T] = 1.0000, [C-G] = 1.0000, [C-T] = 4.6760, [G-T] = 1.0000. These parameters correspond to the general time reversible substitution model and were used for ML calculations.

The resulting ML tree is shown in Fig. 1. The MP tree showed an identical topology. Significant differences in the NJ topology only regarded *Mantella cowani* and *M. haraldmeieri* which changed position in the NJ tree.

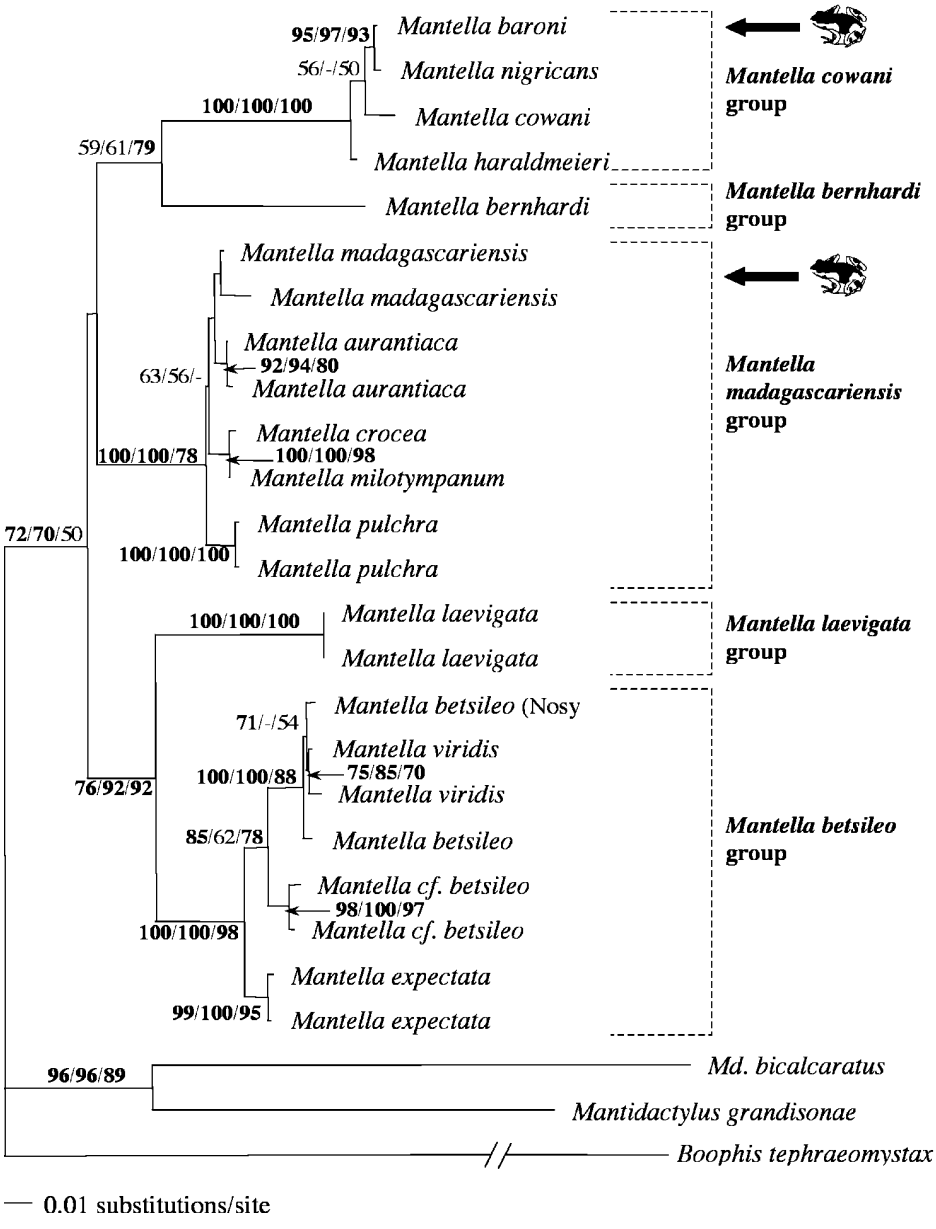

Fig. 1. Maximum Likelihood (general time reversible model) tree of 14 species of *Mantella* based on 1130 bp of the mitochondrial 16S rRNA gene; numbers indicate bootstrap values for 2000 replicates of MP and NJ analyses, and for 100 replicates in ML analysis; only values ≥50% are shown, with those ≥70% printed in boldface. Five well supported groups correspond to the species groups of Vences et al. (1999): the *M. laevigata, M. betsileo, M. madagascariensis, M. cowani* and *M. bernhardi* groups; the *M. aurantiaca* group of Vences et al. (1998) is herein subsumed within the *M. madagascariensis* group according to the tree topology. Arrows indicate the positions of the similar-looking *M. madagascariensis* and *M. baroni*. The branch of the outgroup *Boophis tephraeomystax* was shortened to allow for a clear presentation of short branches.

Sequences of conspecific specimens clustered together in all cases with the exception of *M. betsileo*. The four investigated specimens form two clearly distinct groups. Additional studies will address this issue. Considering only ML-bootstrap values ≥70, a number of well defined clades were apparent which largely corresponded to the species groups as defined by Vences et al. (1999). An exception was the *M. aurantiaca* group which was nested within the *M. madagascariensis* group. Based on the low differentiation of the taxa involved we propose to subsume the *M. aurantiaca* group and the *M. madagascariensis* group. This redefinition will be applied below. Deeper splits among *Mantella* lineages were largely unresolved, except the highly supported placement of *M. laevigata* as sister clade of the *M. betsileo* group, and the weakly supported placement of *M. bernhardi* as a sister clade of the *M. cowani* group.

Mantella baroni and *M. madagascariensis*, though extremely similar in colour pattern, clearly did not resolve as sister species. Their affiliation to different species groups was supported by high bootstrap values of 78–100% in all analyses. Their respective sister species were *M. nigricans* and *M. aurantiaca*. Alternative topologies – (1) placing *M. baroni* as a sister species of *M. madagascariensis* or in any other position within the *M. madagascariensis* group, (2) placing *M. madagascariensis* as sister species of *M. baroni* or in any other position within the *M. cowani* group, or (3) placing both *M. baroni* and *M. madagascariensis* in various basal positions to the *M. madagascariensis* or *M. baroni* groups – were significantly worse than the ML topology (Fig. 1) as revealed by KH tests ($P < 0.005$).

Table 1 shows the ML distances and the absolute number of substitutions between and within the five species groups. Within species groups, mean ML distances ranged from 0.01280 (13.3 substitutions) in the *M. cowani* group to 0.03444 (32 substitutions) in the *M. betsileo* group. Combined, all within-group comparisons yielded a mean ML distance of 0.02217 and a mean of 21.5 substitutions. Among species groups the lowest differentiation was found between the *M. betsileo* and the *M. laevigata* groups. Their mean ML distance was 0.12484 (89.3 substitutions). At the upper end of the range, the *M. laevigata* group was separated from the species of the *M. cowani* group by 123.8 substitutions and a ML distance of 0.20127 on average. The mean ML

Table 1. Pairwise ML distances and absolute numbers of substitutions within and between *Mantella* species groups. Mean ML distance and standard deviation (s) for each pair are displayed in the lower left section of the matrix, values for absolute number of substitutions in the upper right section. Cells on the diagonal show the within-group results (in boldface). One specimen of each species was included in the comparisons. Within-group values are not applicable (n.a.) for the *M. bernhardi* and *M. laevigata* groups which contain only one species. The low level of interspecific genetic differentiation within species groups is clearly distinguishable from the level of differentiation between species from different groups, which is at least three times higher.

Mantella **species group**	***cowani*** **group**		***bernhardi*** **group**		***madagascariensis*** **group**		***laevigata*** **group**		***betsileo*** **group**	
comprising the species	*M. baroni* *M. cowani* *M. haraldmeieri* *M. nigricans*		*M. bernhardi*		*M. aurantiaca* *M. crocea* *M. madagascariensis* *M. milotympanum* *M. pulchra*		*M. laevigata*		*M. betsileo* *M. expectata* *M. viridis*	
	mean	**s**	**mean**	**s**	**mean**	**s**	**mean**	**s**	**mean**	**s**
cowani **group**	**13.3** **0.01280**	**4.63** **0.00461**	103	3.2	103.7	3.2	123.8	3.2	104.5	3.2
bernhardi **group**	0.16282	0.00786	**n.a.** **n.a.**	**n.a.** **n.a.**	106	1.4	115	0	111.3	6.1
madagascariensis **group**	0.15843	0.00745	0.16883	0.00485	**19.1** **0.01927**	**6** **0.00624**	99	1.6	91.3	3.4
laevigata **group**	0.20127	0.00917	0.19027	0	0.14799	0.00367	**n.a.** **n.a.**	**n.a.** **n.a.**	89.3	3.5
betsileo **group**	0.16302	0.0068	0.19344	0.02759	0.13786	0.00424	0.12484	0.00454	**32** **0.03444**	**3.0** **0.00534**

distance of all between-group comparisons was 0.16487 or 104.7 substitutions. The levels of genetic differentiation between *M. madagascariensis, M. baroni* and their respective sister species *M. nigricans* and *M. aurantiaca* are shown in Table 2. The mean distances between the sister species were 0.00460 (5 substitutions) and 0.01177 (12.5 substitutions), respectively. In contrast, the genetic distance between *M. baroni* and *M. madagascariensis* was an order of magnitude higher: 0.15751 (105.5 substitutions).

The number of substitutions between conspecific specimens ranges from 0 (for the *M. laevigata* specimens) to 13 (for the *M. madagascariensis* specimens) with a mean of 4.3. This corresponds to ML distances from 0 to 0.01221 (mean: 0.00405). *M. betsileo* specimens were excluded from this comparison because they were not monophyletic.

Discussion

The investigation of the levels of genetic differentiation within *Mantella* yielded a well supported and consistent pattern of five species groups. Differentiation among these species groups is pronounced, with ML distances ranging from 0.11965 to 0.22492. Within-group differentiation tends to be less regular and very low in some groups. Nevertheless, the range of ML distances (0.00371-0.03922) is clearly distinct from the between-groups values. The minimum distances within the *M. baroni* and the *M. madagascariensis* groups were as low as the mean distance between conspecific specimens. The mean ML distance within the *M. baroni* group (0.01280) was almost as low as the highest conspecific distance (*M. madagascariensis*: 0.01221). *Mantella baroni* and *M. madagascariensis* showed very low distances to their morphologically different sister species. Apparently, factors existed in *Mantella* evolution which led to sudden and comprehensive changes in dorsal body coloration.

The fact that the resolution of basal splits among *Mantella* species groups remains ambiguous may indicate a very rapid basal radiation of *Mantella* with not enough time for the acquisition of an adequate amount of synapomorphic character states along internal branches. The second level of differentiation (within species groups) may be indicative of a second radiation in at least some groups.

Our data show that two species with striking similarity in coloration and pattern (*M. baroni* and *M. madagascariensis*) clearly are not sister species but belong to two genetically distinct species groups. This result is supported by Vences et al. (1998b, c) who placed the two species into different groups based on allozyme and osteological data.

Mantella madagascariensis and *M. baroni* are distinguished by different morphology and ventral coloration, but they share the following detailed patterns of dorsal coloration: (1) large yellow-greenish flank blotches (otherwise present only in *M. pulchra* and *M. nigricans*), (2) yellow-greenish rostral stripes (not present in other species), (3) deep black dorsal colour including head surface (otherwise only in *M. cowani*), (4) orange hindlimbs with black crossbands (not present in other species), (5) yellow lower forelimbs (otherwise only in *M. aurantiaca, M. milotympanum, M. crocea*). *Mantella aurantiaca*, the sister species of *M. madagascariensis*, is entirely orange or reddish. *Mantella baroni* clustered with *M. haraldmeieri*, *M. nigricans* and *M. cowani*, and shares with these species the colourful flank blotches, but never the exact pattern (see colour plates in Electr. Suppl. 02–04, Pt 1).

Three contrasting hypotheses may account for the striking similarity in the dorsal colour pattern of *M. madagascariensis* and *M. baroni*: (1) the two species are sister taxa that share a synapomorphic dorsal pattern; (2) the two species retained plesiomorphies which account for the similarities; or (3) their identical pattern is a case of homoplastic evolution. Clearly, hypothesis 1 is not supported by our phylogenetic analyses. It is more difficult to exclude the second hypothesis. Similar general patterns are also found in other related species within *Mantella*. However, it must be kept in mind that many specimens of *M. madagascariensis* and *M. baroni* have a virtually identical dorsal pattern and are sometimes not even distinguishable by ventral pattern. The pattern and colour similarity is therefore not only a general one, but

Table 2. Pairwise ML distances and absolute numbers of substitutions between *M. madagascariensis, M. baroni*, and their respective sister species. Within each species row, the upper line contains the mean absolute number of differences and the corresponding standard deviation (s) for each pair, the lower line gives the values for ML distance. All available specimens (2 *M. madagascariensis*, 2 *M. aurantiaca*, 1 *M. baroni*, 1 *M. nigricans*) were included in the comparisons. The respective levels of genetic differentiation – between *M. baroni* and its sister species, *M. nigricans,* and between *M. madagascariensis* and its sister species, *M. aurantiaca* – was very low. The level of genetic differentiation between the similar-looking *M. baroni* and *M. madagascariensis* was 9 to 21 times higher.

Species	*M. madagascariensis*		*M. nigricans*	
	mean	s	mean	s
M. baroni	105.5 0.15751	0.7 0.00063	5 0.00460	_ _
M. aurantiaca	12.5 0.01177	4.8 0.00467	106.5 0.16380	0.7 0.00036

extends to at least five characters as listed above. To consider these largely identical character states symplesiomorphies would imply their multiple loss or modification in several independent lineages according to the phylogeny reconstructed here. Homoplastic evolution in both species therefore is the most parsimonious hypothesis. This hypothesis would be even better supported if the sister group relationship of *Mantella bernhardi* to the *M. cowani* group and the basal position of *M. haraldmeieri* in this group were confirmed by additional data. These two species show a pattern which at least partly must be regarded as cryptic, and their present position in the tree (Fig. 1) indicates that this pattern may have been ancestral for the *M. cowani* group.

In combination with published data (Vences et al. 1998b, c; Pintak et al. 1998), and considering recent progress in the understanding of juvenile coloration (Glaw et al. 2000), a scenario of pattern evolution within *Mantella* can be inferred. Most splits in the assumed phylogeny (Fig. 2) are also supported by osteology, karyology and allozymes. Species of the sister group of *Mantella* (the *Mantidactylus* subgenus *Blommersia*) have a brownish back, sometimes slightly darker flanks, and a white frenal stripe (Glaw & Vences 1994). *Mantella betsileo* largely retains this pattern, except with deep black flanks and light forelimbs. This *M. betsileo*-like coloration is expressed by recently metamorphosed young of all *Mantella* species and is here considered as plesiomorphic (hypothetical ancestor 1 in Fig. 2). Colourful legs may have evolved independently in different species groups (hypothetical ancestors 2 and 3). Subsequently, totally or partially black legs, as in *M. laevigata* and *M. cowani*, evolved homoplastically. The plesiomorphic light dorsal coloration presumably was still present in ancestor 3. We assume that in ancestor 4 dorsal coloration was still lighter than the black flanks but very faintly so, giving the impression of an overall blackish body as in *M. bernhardi*. Flank blotches above arms and legs were present as small spots in ancestors 3 and 4 (as currently is seen in *M. bernhardi* and *M. haraldmeieri*), and homoplastically became larger in species of the *M. madagascariensis* group and the *M. cowani* group. Thus, several general patterns shared by *M. madagascariensis* and *M. baroni* (flank blotches, colourful legs) were presumably already expressed to lesser degrees in their most recent common ancestor but were modified homoplastically to match their present extension and colour.

As Glaw & Vences (2000) did before, we consider Müllerian mimicry as one probable explanation for the

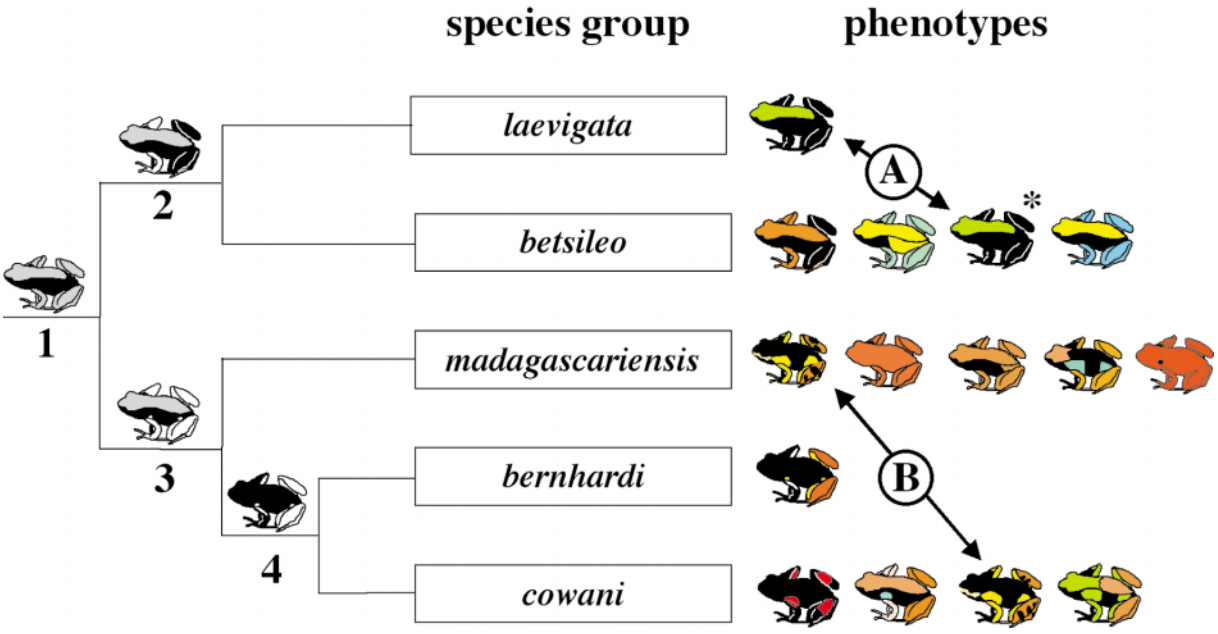

Fig. 2. Hypothesis on the evolution of aposematic patterns among *Mantella* species groups. Phylogeny is based on molecular data as presented in Fig. 1; and on osteological data and allozyme differentiation (Vences et al. 1998b, c). The asterisk indicates *M. manery* which has been included in the *M. betsileo* group by Vences et al. (1999) based on morpho-chromatic characters. Phenotypes at internal branches illustrate the assumed plesiomorphic pattern of hypothetical ancestors 1–4 as discussed in the text; arrows indicate species pairs of assumed homoplastic pattern evolution: A = *M. laevigata*/*M. manery*, B = *M. madagascariensis*/*M. baroni*.

pattern convergence seen in *Mantella*. So far, only very few examples of Müllerian mimicry in amphibians have been described. A potential case among amphibians comprises the unpalatable salamanders *Pseudotriton ruber* and *P. montanus* which mimic the noxious red eft stage of *Notophthalmus viridescens* (Howard & Brodie 1971, 1973; Brodie & Howard 1972; Brandon et al. 1979; Brandon & Huheey 1981). Symula et al. (2001) provide evidence for a case of Müllerian mimetic radiation in Peruvian poison frogs.

In *Mantella*, several characteristics support our assumption. (1) Their coloration is considered aposematic. (2) Their skin contains alkaloid toxins. (3) *Mantella* species are largely diurnal and such species are more likely to show mimicry. Avoidance of special colours and patterns by predators is best learned by day, when the signals are perceptible to visual predators. (4) Field data on pattern variation meet three predictions that can be made about variation of aposematic coloration under Müllerian mimicry (Mallet & Joron 1999): (i) pattern identity of the two species is high and stable in syntopy (stabilising selection); (ii) stabilised patterns may be different in different localities of syntopy, since frequency dependence of selection under Müllerian mimicry can occasionally make the mimic the model; and (iii) pattern variation of allotopic populations will be higher than in syntopic ones.

Although the overlap of the range of *M. madagascariensis* with that of *M. baroni* (degree of overlap of the minimum convex polygons spanned by known localities) is almost 100%, syntopy of both is only known from two localities: Vohiparara, near the Ranomafana National Park, and Niagarakely, both in south-eastern Madagascar (Vences et al. 1999). At these localities, specimens of both taxa look almost identical (molecular data presented herein refer to syntopic specimens from Vohiparara). It is not known whether the 'variable morph' of *M. madagascariensis*, in which the yellowish and greenish colour may even cover parts of the dorsum, occurs in syntopy with *M. baroni*. However, while *M. baroni* from most populations are uniform in dorsal colour and pattern (Glaw & Vences 2000), at Ivohibe, south of the known distribution of *M. madagascariensis*, an enigmatic population similar to *M. baroni* exists which displays remarkable pattern variability, including almost completely yellow specimens (Vences et al. 1999). This pattern variation is in full concordance with predictions (i) and (iii).

Our data provide evidence for homoplastic pattern evolution in *M. madagascariensis* and *M. baroni,* and the same phenomenon may apply to *M. laevigata* and *M. manery* Vences, Glaw & Böhme, 1999 (Fig. 2). In contrast, the existence of Müllerian mimicry within *Mantella* remains to be demonstrated. In addition to a fully resolved phylogeny, this would require proof of aposematism, the detection of toxins in both species, and the verification of unpalatability for their enemies. Finally, predator learning and selective prey choice by predators should also be tested before the mimicry hypothesis can be considered as sufficiently corroborated.

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References

Andreone, F. (1992): Syntopy of *Mantella cowani* Boulenger and *Mantella madagascariensis* (Grandidier) in central-eastern Madagascar, with notes on the coloration in the genus *Mantella* (Anura: Mantellidae). Boll. Mus. Reg. Sci. Nat. Torino 10: 421–450.

Brandon, R. A. & Huheey, J. E. (1981): Toxicity in the plethodontid salamanders *Pseudotriton ruber* and *Pseudotriton montanus* (Amphibia, Caudata). Toxicon 19: 25–31.

Brandon, R. A., Labanick, G. M. & Huheey, J. E. (1979): Relative palatability, defensive behavior and mimetic relationships of red salamanders (*Pseudotriton ruber),* mud salamanders (*Pseudotriton montanus*), and red efts (*Notophthalmus viridescens*). Herpetologica 35: 289–303.

Brodie, E. D., jr. & Howard, R. R. (1972): Behavioral mimicry in the defensive displays of the urodele amphibians *Notophthalmus viridescens* and *Pseudotriton ruber.* BioScience 22: 666–667.

Busse, K. & Böhme, W. (1992): Two remarkable frog discoveries of the genera *Mantella* (Ranidae: Mantellinae) and *Scaphiophryne* (Microhylidae: Scaphiophryninae) from the west coast of Madagascar. Revue Fr. Aquariol. 19: 57–64.

Caldwell, J. P. (1996): The evolution of myrmecophagy and its correlates in poison frogs (Family Dendrobatidae). J. Zool. 240: 75–101.

Daly, J. W., Andriamaharavo, N. R., Andriantsiferana, M. & Myers, C. W. (1996): Madagascan poison frogs (*Mantella*) and their skin alkaloids. Amer. Mus. Novitates 0(3177): 1–34.

Daly, J. W., Myers, C. W. & Whittaker, N. (1987): Further classification of skin alkaloids from neotropical poison frogs (Dendrobatidae), with a general survey of toxic/noxious substances in the amphibia. Toxicon 25: 1023–1095.

Daly, J. W., Secunda, S. I., Garraffo, H. M., Spande, T. F., Wisnieski, A. & Cover, J. F., jr. (1994): An uptake system for dietary alkaloids in poison frogs (Dendrobatidae). Toxicon 32: 657–663.

Felsenstein, J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Glaw, F. & Vences, M. (1994): A Fieldguide to the Amphibians and Reptiles of Madagascar. Second Edition – Including Mammals and Freshwater Fish. 480 pp., Vences & Glaw, Cologne.

Glaw, F. & Vences, M. (2000): *Mantella madagascariensis* und *Mantella baroni*. D. Aquar.-Terr. Z. 53: 18–22.

Glaw, F., Vences, M. & Schmidt, K. (2000): Nachzucht, Juvenilfärbung und Oophagie von *Mantella laevigata* im Vergleich zu anderen Arten der Gattung (Amphibia: Ranidae). Salamandra 36: 1–24.

Hamilton, W. D. & Zuk, M. (1982): Heritable true fitness and bright birds: a role for parasites? Science 218: 384–387.

Hedges, S. B. (1992): The number of replications needed for accurate estimation of the bootstrap P-value in phylogenetic studies. Mol. Biol. Evol. 9: 366–369.

Hedges, S. B. (1994): Molecular evidence for the origin of birds. Proc. Natl Acad. Sci. U.S.A. 91: 2621–2624.

Howard, R. R. & Brodie, E. D. (1971): Experimental study of mimicry in salamanders involving *Notophthalmus viridescens viridescens* and *Pseudotriton ruber schenki*. Nature 233: 277.

Howard, R. R. & Brodie, E. D. (1973): A Batesian mimetic complex in salamanders: responses of avian predators. Herpetologica 29: 33–41.

Huelsenbeck, J. P. & Hillis, D. M. (1993): Success of phylogenetic methods in the four-taxon case. Syst. Biol. 42: 247–264.

Kishino, H. & Hasegawa, M. (1989): Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominidea. J. Mol. Evol. 29: 170–179.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. & Wilson, A. C. (1989): Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. Proc. Natl Acad. Sci. U.S.A. 86: 6196–6200.

Lockhart, P. J., Steel, M. A., Hendy, M. D. & Penny, D. (1994): Recovering evolutionary trees under a more realistic model of sequence evolution. Mol. Biol. Evol. 11: 605–612.

Mallet, J. & Joron, M. (1999): Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. Annu. Rev. Ecol. Syst. 30: 201–233.

Mausfeld, P., Vences, M., Schmitz, A. & Veith, M. (2000): First data on the molecular phylogeography of scincid lizards of the genus *Mabuya*. Mol. Phyl. Evol. 17: 11–14.

Palumbi, S., Martin, A., Romano, S., McMillan, W. O., Stice, L. & Grabowski, G. (1991): The Simple Fools Guide to PCR. Version 2.0, Dept. Zool. and Kewalo Marine Lab., Univ. of Hawai'i.

Pintak, T. & Böhme, W. (1988): *Mantella viridis* sp. n. aus Nord-Madagaskar. Salamandra 24: 119–124.

Pintak, T. & Böhme, W. (1990): *Mantella crocea* sp. n. (Anura: Ranidae: Mantellinae) aus dem mittleren Ost-Madagaskar. Salamandra 26: 58–62.

Pintak, T., Vences, M., Glaw, F. & Böhme, W. (1998): Comparative chromosome morphology of Malagasy poison frogs (Amphibia: Ranidae: *Mantella*). Folia Zool. 47: 197–204.

Posada, D. & Crandall, K. A. (1998): MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Servedio, M. R. (2000): The effects of predator learning, forgetting, and recognition errors on the evolution of warning coloration. Evolution 54: 751–763.

Summers, K. & Clough, M. E. (2001): The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). Proc. Natl Acad. Sci. U.S.A. 98: 6227–6232.

Swofford, D. L. (2001): PAUP*. Phylogenetic analysis using parsimony (*and other methods), ver. 4.0b8. Sinauer, Sunderland, Massachusetts.

Symula, R., Schulte, R. & Summers, K. (2001): Molecular phylogenetic evidence for a mimetic radiation in Peruvian poison frogs supports a Müllerian mimicry hypothesis. Proc. R. Soc. Lond. B 268: 2415–2421.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997): The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. 24: 4876–4882.

Vences, M. & Glaw, F. (2001): When molecules claim for taxonomic change: new proposals on the classification of Old World treefrogs. Spixiana 24: 85–92.

Vences, M., Glaw, F. & Böhme, W. (1998a): Evolutionary correlates of microphagy in alkaloid-containing frogs (Amphibia: Anura). Zool. Anz. 236: 217–230.

Vences, M., Glaw, F. & Böhme, W. (1999): A review of the genus *Mantella* (Anura, Ranidae, Mantellinae): taxonomy, distribution and conservation of Malagasy poison frogs. Alytes 17: 3–72.

Vences, M., Glaw, F., Mausfeld, P. & Böhme, W. (1998b): Comparative osteology of Malagasy poison frogs of the genus *Mantella* (Amphibia: Ranidae: Mantellinae). Bonner Zool. Beitr. 48: 205–215.

Vences, M., Hille, A. & Glaw, F. (1998c): Allozyme differentiation in the genus *Mantella* (Amphibia: Anura: Mantellinae). Folia Zool. 47: 261–274.

Vences, M., Kosuch, J., Lötters, S., Widmer, A., Köhler, J., Jungfer, K.-H. & Veith, M. (2000): Phylogeny and classification of poison frogs (Amphibia: Dendrobatidae), based on mitochondrial 16S and 12S ribosomal RNA gene sequences. Mol. Phyl. Evol. 15: 34–40.

Appendix

Specimens examined and GenBank accession numbers. Abbreviations: ZFMK = Zoologisches Forschungsinstitut und Museum Alexander Koenig, Germany; n.p. = voucher not preserved; l.u. = locality unknown, specimen obtained through the pet trade.

Species	**Locality**	**Collection no.**	**GenBank accession no. 16S rRNA sequence**	
			1st section	**2nd section**
Boophis tephraeomystax (Duméril, 1853)	Isalo	ZFMK 70495	AF215332	AJ438907
Mantidactylus bicalcaratus (Boettger, 1913)	Masoala	ZFMK 72048	AF215323	AJ438906
Mantidactylus grandisonae Guibé, 1974	Ambato	ZFMK 66669	AF215149	AJ438905
Mantella aurantiaca Mocquard, 1900	l.u.	ZFMK 62776	AF215299	AJ438895
Mantella aurantiaca Mocquard, 1900	l.u.	ZFMK 72143	AF215298	AJ438894
Mantella baroni Boulenger, 1888	Vohiparara	ZFMK 72146	AF215302	AJ438901
Mantella bernhardi Vences et al., 1994	l.u.	ZFMK 62698	AF215311	AJ438896
Mantella betsileo (Grandidier, 1872)	Anove	ZFMK 70484	AF215283	AJ438886
Mantella betsileo (Grandidier, 1872)	Kirindy	ZFMK 66695	AF215281	AJ438884
Mantella betsileo (Grandidier, 1872)	Nosy Be	ZFMK 62688	AF215282	AJ438885
Mantella betsileo (Grandidier, 1872)	Morondava	n.p.	AF215288	AJ438887
Mantella cowani Boulenger, 1882	l.u.	ZFMK 62731	AF215305	AJ438903
Mantella crocea Pintak & Böhme, 1990	l.u.	ZFMK 62767	AF215309	AJ438897
Mantella expectata Busse & Böhme, 1992	l.u.	n.p.	AF215295	AJ438889
Mantella expectata Busse & Böhme, 1992	l.u.	ZFMK 62789	AF215296	AJ438888
Mantella haraldmeieri Busse, 1981	l.u.	n.p.	AF215312	AJ438904
Mantella laevigata Methuen & Hewitt, 1913	l.u.	n.p.	AF215279	AJ438588
Mantella laevigata Methuen & Hewitt, 1913	l.u.	ZFMK 65637	AF215280	AJ438589
Mantella madagascariensis (Grandidier, 1872)	Vohiparara	ZFMK 64138	AF215301	AJ438893
Mantella madagascariensis (Grandidier, 1872)	l.u.	ZFMK 72148	AF215300	AJ438892
Mantella milotympanum Staniszewski, 1996	l.u.	n.p.	AF215310	AJ438898
Mantella nigricans Guibé, 1978	l.u.	ZFMK 72015	AF215308	AJ438902
Mantella pulchra Parker, 1925	l.u.	ZFMK 62742	AF215307	AJ438900
Mantella pulchra Parker, 1925	l.u.	ZFMK 72142	AF215306	AJ438899
Mantella viridis Pintak & Böhme, 1988	l.u.	n.p.	AF215292	AJ438890
Mantella viridis Pintak & Böhme, 1988	l.u.	ZFMK 72145	AF215293	AJ438891