

## Karyological Divergence in three Platymantine Frogs, Family Ranidae

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**Abstract.** The karyotypes of three platymantine frogs, *Platymantis hazelae* and *P. dorsalis* from Philippines and *P. papuensis* from Papua New Guinea, were described and compared. *P. hazelae* has  $2n = 26$  chromosomes consisting of five large and eight small pairs, as in many other species of Ranidae. The karyotypes of *P. dorsalis* ( $2n = 20$ ) and *P. papuensis* ( $2n = 22$ ) may be derived from the basic karyotype of  $2n = 26$ . Simple fusions and pericentric inversions explain the karyotypic relationships between *P. hazelae* and *P. papuensis*, while the karyotype of *P. dorsalis* seems to have resulted from more complicated chromosome rearrangements.

### Introduction

The animals occurring in a series of fringing islands off the east coast of Asia and Australia provide favorable materials for the studies of speciation. There is enough evidence that evolutionary changes may be accelerated in island populations due to factors associated with small population size and founder effect (Mayr, 1963; Dobzhansky et al., 1977). Animals without ability to cross the sea water, such as amphibians, have been effectively isolated in these islands and thus are the most preferable materials for such speciation studies.

The genus *Platymantis* in the frog family Ranidae, subfamily Platymantinae, occurs in the Philippines, Palau, New Guinea, Bismarcks, Solomons and Fijis (Brown, 1952; Inger, 1954; Gorham, 1965). This genus is well known for its direct development (Inger, 1954; Alcalá, 1962). Excepting the cosmopolitan genus *Rana*, *Platymantis* is the largest genus among Ranidae in the Oriental and Australian regions. It involves 37 species (Brown and Alcalá, 1974; Gorham, 1974; Menzies, 1982), most of which are endemic to one or a few islands. Although *Platymantis* has diverged so extensively in these islands, the inter-species relationships have not been examined in detail.

In this study, I examined karyotypes of three platymantine frogs and inferred species relationships based on karyotypic comparisons. Karyotypes can provide information pertaining to species relationships (Bogart and Nelson, 1976; Morescalchi et

al., 1977; Bogart and Tandy, 1981). Several ranid frogs of the Ryukyu Islands have karyotypes very similar to each other, and hence are presumed to have a common origin (Kuramoto, 1972). In some cases where two congeneric species have different chromosome numbers, the karyotype of one species may be derived from that of the other through rather simple structural changes of chromosomes (Menzies and Tippett, 1976).

### Materials and Methods

The three species examined were: *Platymantis hazelae* (4 ♀ ♀, 3 ♂ ♂) and *P. dorsalis* (2 ♀ ♀, 3 ♂ ♂) from the vicinity of Lake Balinsasayao, Negros Oriental, Philippines and *P. papuensis* (2 ♀ ♀, 4 ♂ ♂) from Lae, Morobe, and Angoram, East Sepik, Papua New Guinea. Chromosome preparations were made from bone marrow cells following Omura's (1967) method. Several metaphase spreads were photographed and relative length (length of each homologous chromosome pair with total length of the haploid set being 100) and arm ratio (long arm length / short arm length) were calculated from the measurements on enlarged photomicrographs. Chromosome pairs were numbered in the order of decreasing mean relative length, and centromeric position was designated according to Levan et al. (1964). The three karyotypes were compared with each other, and the significance of species differences in corresponding chromosome pairs were tested (*t*-test). It was then determined whether simple structural changes in chromosomes of one species could produce karyotypes similar to those of the others.

### Results

*P. hazelae* had 13 pairs of biarmed chromosomes consisting of five large and eight small pairs (Fig. 1A). Pair nos. 2, 3, 4, 7, 9 and 13 were submedian and the other seven pairs median (Table 1). Pair nos. 3 and 4, nos. 6 and 7, nos. 9 and 10, nos. 10 and 11, and nos. 11 and 12 did not differ significantly in relative length, but in the first three of these combinations arm ratio differed significantly ( $P < .05$ ). No secondary constrictions nor heteromorphic pairs were observed.

*P. dorsalis* had 10 pairs of biarmed chromosomes which could not be classified into distinct size groups (Fig. 1B). Pair no. 2 was subterminal, no. 4 submedian, and the other eight median (Table 1). Pair no. 9 had a secondary constriction on the short arm. All chromosome pairs differed significantly in relative length ( $P < .05$ ). I could not find heteromorphic pairs.

*P. papuensis* had 11 pairs of biarmed chromosomes which could not be classified into distinct size groups (Fig. 1C). Since the materials from Lae and Angoram gave essentially identical results, the values for the two populations are pooled in Table 1. Pair nos. 2, 3, 4, 7 and 9 were submedian and the other six pairs median. Pair nos. 3 and 4 and nos. 9 and 10 did not differ in relative length but they were readily distinguishable by the differences in arm ratio. No secondary constrictions nor heteromorphic pairs were observed.



Fig. 1. Karyotypes of *P. hazelae* (A), *P. dorsalis* (B), and *P. papuensis* (C). Scales equal 10  $\mu$ m.

Table 1. Relative length and arm ratio of the chromosome pairs in three platymantine frogs ( $\bar{x} \pm$  SE).

pair no.	<i>P. hazelae</i> (N = 11)		<i>P. dorsalis</i> (N = 11)		<i>P. papuensis</i> (N = 20)	
	relative length	arm ratio <sup>a</sup>	relative length	arm ratio <sup>a</sup>	relative length	arm ratio <sup>a</sup>
1	15.6 $\pm$ .24	1.27 $\pm$ .02 m	17.4 $\pm$ .36	1.08 $\pm$ .01 m	16.0 $\pm$ .16	1.26 $\pm$ .01 m
2	12.9 $\pm$ .18	1.70 $\pm$ .03 sm	13.0 $\pm$ .21	4.19 $\pm$ .27 st	13.2 $\pm$ .13	1.89 $\pm$ .03 sm
3	11.2 $\pm$ .26	2.17 $\pm$ .03 sm	12.3 $\pm$ .13	1.27 $\pm$ .03 m	11.6 $\pm$ .13	2.27 $\pm$ .06 sm
4	10.8 $\pm$ .12	1.83 $\pm$ .05 sm	11.6 $\pm$ .17	2.77 $\pm$ .10 sm	11.3 $\pm$ .14	1.90 $\pm$ .05 sm
5	9.9 $\pm$ .09	1.33 $\pm$ .02 m	10.1 $\pm$ .15	1.25 $\pm$ .03 m	10.2 $\pm$ .10	1.38 $\pm$ .02 m
6	5.8 $\pm$ .12	1.33 $\pm$ .03 m	8.6 $\pm$ .14	1.12 $\pm$ .02 m	9.1 $\pm$ .06	1.60 $\pm$ .03 m
7	5.7 $\pm$ .12	2.40 $\pm$ .06 sm	7.8 $\pm$ .10	1.15 $\pm$ .04 m	7.6 $\pm$ .10	1.89 $\pm$ .05 sm
8	5.3 $\pm$ .08	1.33 $\pm$ .04 m	7.3 $\pm$ .11	1.12 $\pm$ .02 m	5.9 $\pm$ .08	1.61 $\pm$ .04 m
9	5.0 $\pm$ .09	2.31 $\pm$ .08 sm	6.8 $\pm$ .16	1.22 $\pm$ .05 m	5.3 $\pm$ .07	2.08 $\pm$ .04 sm
10	4.9 $\pm$ .08	1.26 $\pm$ .03 m	5.1 $\pm$ .13	1.14 $\pm$ .02 m	5.2 $\pm$ .07	1.42 $\pm$ .03 m
11	4.7 $\pm$ .08	1.20 $\pm$ .05 m			4.5 $\pm$ .05	1.26 $\pm$ .02 m
12	4.5 $\pm$ .09	1.24 $\pm$ .04 m				
13	3.8 $\pm$ .09	2.99 $\pm$ .15 sm				

a: m, sm and st are abbreviations of median, submedian and subterminal.

The karyotypes of *P. hazelae* and *P. papuensis*, although their chromosome numbers differed, can be correlated with each other. As shown in Figure 2 and Table 1, the large chromosome pairs of the two are very similar in size and shape. Also, there are several small chromosome pairs that resemble each other. If nos. 6 and 7 of *P. papuensis* are assumed to split at the position of the centromere, the resulting four arms correspond fairly well to some small chromosomes of *P. hazelae* in relative length. It was demonstrated by *t*-test that nos. 1, 3, 4, 5 and 11 of the two species did not differ

significantly both in relative length and in arm ratio. Pair no. 8 of *P. hazelae* and no. 10 of *P. papuensis* also did not differ in both parameters. Long arms of nos. 6 and 7 of *P. papuensis* did not differ significantly from relative length of nos. 7 and 10 of *P. hazelae*. Pair nos. 2 and 9 of the two species, as well as no. 6 of *P. hazelae* and no. 8 of *P. papuensis*, differed in their arm ratio, but did not differ in their relative length. Thus, it is apparent that two centric fissions in two medium-sized pairs of *P. papuensis* and subsequent pericentric inversions produce a karyotype very similar to that of *P. hazelae* (Fig. 2). The chromosomal changes in the reverse direction can produce the karyotype of *P. papuensis* from that of *P. hazelae*.

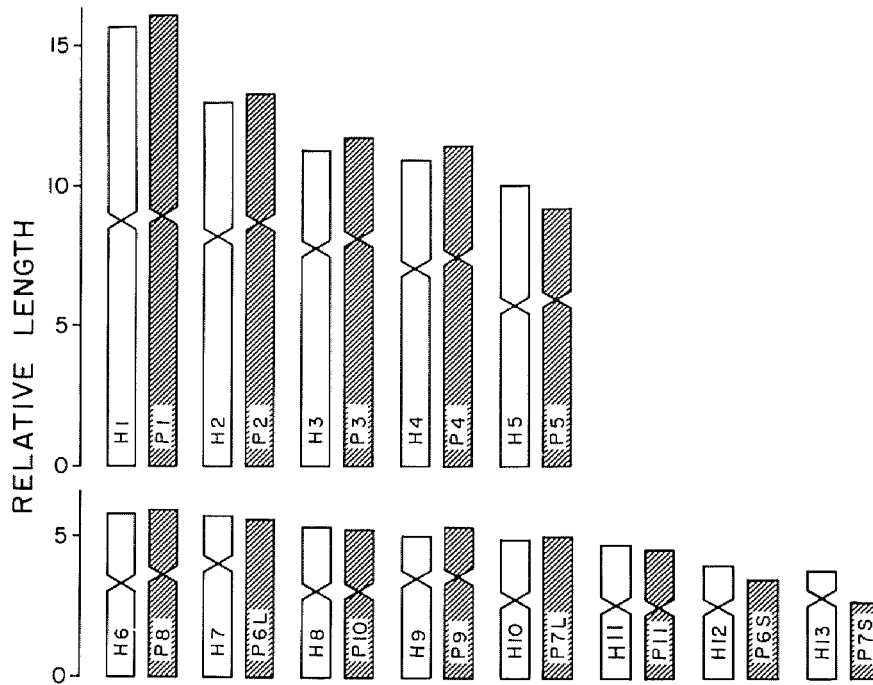


Fig. 2. A comparative idiogram showing karyotypic correlations between *P. hazelae* and *P. papuensis*. H and P. indicate chromosome pairs of *P. hazelae* and *P. papuensis*, figures indicate pair no., and L and S indicate long arm and short arm.

In contrast, I could not reconstruct a karyotype similar to that of *P. hazelae* or *P. papuensis* from that of *P. dorsalis*. None of the chromosome pairs of *P. dorsalis* correspond to any one pair of *P. hazelae* and *P. papuensis* in both relative length and arm ratio ( $P < .05$ ), except for no. 5 of *P. dorsalis* and *P. hazelae*. To derive the karyotype of *P. hazelae* or *P. papuensis* from that of *P. dorsalis*, therefore, a complicated sequence of chromosomal changes is required. In one of my attempts to produce the *hazelae*-like karyotype, the following changes were needed; centric fissions in six chromosome pairs

of *P. dorsalis*, fusions in six of the resultant 12 arms, translocations in two pairs, and pericentric inversions in five pairs. The karyotype thus obtained, however, was not so similar to the karyotype of *P. hazelae* as the transformed karyotype of *P. papuensis*.

### Discussion

The amphibian fauna of the Philippines is mostly composed of the Oriental elements; *Platymantis* and a microhylid genus *Oreophryne* which are thought to be New Guinean elements are exceptions (Inger, 1954). Because only one *Oreophryne* species occurs in southern Philippines (Mindanao), in contrast to 13 in New Guinea (Zweifel and Tyler, 1982), the New Guinean origin of *Oreophryne* is inferred. Species diversity in the genus *Platymantis*, however, does not so clearly support its New Guinean origin. Currently 11 species are reported to occur in the Philippines (Luzon to Mindanao), one in Palau, four in New Guinea and adjacent small islands, 10 in Bismarcks, 10 in Solomons, and two in Fijis (Brown, 1965; Gorham, 1965; Zweifel, 1969; Brown and Alcala, 1970, 1974; Brown and Menzies, 1979; Menzies, 1982; Zweifel and Tyler, 1982). This species diversity suggests that *Platymantis* has two major radiation centers, one in the Philippines and the other in the Bismarcks and Solomons. Of three species occurring on the New Guinea mainland, *P. papuensis* occurs widely along northern coastal region and the other two are known only from their type localities (Zweifel, 1969). Although the New Guinean fauna has not been thoroughly studied as yet, the poverty of *Platymantis* species in New Guinea is remarkable, and this seems to argue against a New Guinean origin of the genus.

The present karyological study supports the hypothesis that the genus *Platymantis* originated in the Philippines and invaded New Guinea. It has been established that the basic karyotype of the family Ranidae consists of  $2n = 26$  chromosomes with five large and eight small pairs (Morescalchi, 1973). Since *P. hazelae* has this basic karyotype, it is highly probable that *P. hazelae* belongs to a primitive stock in this genus. On the other hand, *P. dorsalis* and *P. papuensis* are derived forms with modified karyotypes. Surveying the anuran chromosome numbers (see Morescalchi, 1973), it is evident that the number tends to be reduced from generalized to derived forms, as well as from primitive to advanced groups. This fact also suggests that *P. dorsalis* and *P. papuensis* are derived forms.

From the karyotypic comparisons discussed above, *P. papuensis* is presumed to originate from a stock that had a karyotype similar to that of *P. hazelae* through pericentric inversions and subsequent chromosomal fusions. Inger (1954) pointed out the morphological similarities of *P. papuensis* and *P. dorsalis*. From their karyotypes, however, these two species can not be regarded as closely related, because there are no simple karyological correlations between the two. Morphological similarity of *P. papuensis* and *P. dorsalis* may reflect a convergence due to their similar mode of life. *P. dorsalis* seems to be a highly specialized form undergoing a series of complicated karyological changes from the basic karyotype.

The above relationships of the three species are based on an assumption of a Philippine origin of the genus and a general trend of chromosomal reduction in anurans. If the hypothesis is valid, the karyotypes of platymantine frogs in the Bismarcks and Solomons are expected to show some characteristics indicating their derivation from that of *P. papuensis*. Further karyological studies with many other platymantine frogs will shed light on the phylogenetic relationships in this genus. Using C-banding methods, Green (1983) inferred the process of chromosome number reduction in some Palearctic brown frogs. Although I failed to obtain banding karyotypes, various methods for chromosome banding (Schmid, 1978, 1980) will reveal more detailed karyological relationships between *Platymantis* species.

Probably, the minor radiation of *Platymantis* in New Guinea is due to an extensive radiation of two microhylid subfamilies there, Asterophryinae and Sphenophryinae, as suggested by Menzies (1976). The two microhylid subfamilies are represented by only one species in the Philippines, two in Bismarcks (New Britain) and none in Solomons, while 74 species occur on the New Guinea mainland (Inger, 1954; Brown and Menzies, 1979; Zweifel and Tyler, 1982). These microhylids have life histories involving direct development and thus may compete severely with *Platymantis* in their ecological requirements. It seems that a secondary radiation of *Platymantis* occurred when the genus arrived the Bismarcks and Solomons where the microhylids are scarce or absent.

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