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Title EXPERIMENTAL HYBRIDIZATION BETWEEN RANA PRETIOSA PRETIOSA BAIRD AND GIRARD AND RANA CASCADAE SLATER

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Experimental crosses between Rana pretiosa pretiosa Baird and Girard and Rana cascadae Slater, resulted in viable hybrids. Reciprocal differences at the time of fertilization are indicated. A high level of success in producing viable hybrids can be obtained if Rana pretiosa pretiosa is the female of the parent species. The hybrid is characteristically intermediate to the parent species.

The hybrid is quantitatively intermediate to the parent species in the characteristics of larval body width/tail depth ratios, developmental time, and mouth parts.

Statistically treated larval body width/tail depth ratios of natural populations can be used to determine the amount and direction of natural hybridization between the species.

EXPERIMENTAL HYBRIDIZATION BETWEEN *RANA PRETIOSA PRETIOSA* BAIRD AND GIRARD AND *RANA CASCADAE* SLATER

by

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EXPERIMENTAL HYBRIDIZATION BETWEEN *RANA PRETIOSA PRETIOSA* BAIRD AND GIRARD AND *RANA CASCADAE* SLATER

INTRODUCTION

Early workers assumed that anuran morphological intermediates occurring in areas of species overlap were the result of natural hybridization. Since 1941, workers have been able to confirm the assumption by experimental hybridization. External fertilization, characteristic of the group, allows the investigator selection of the gametes for fusion, and the resulting hybrid can then be compared to known ancestry (1, 2, 13, 29).

In recent years, experimental hybridization in addition to other techniques (3, 6, 13, 18) has gained impetus as evidence for judgements of anuran relationships. Bufonids and hylids are the two groups which have received the most attention (13, 16, 17, 29, 30, 32, 33), whereas frogs belonging to the genus *Rana* have received somewhat less (6, 19, 22, 34).

Studies of natural hybridization relate to questions of isolating mechanisms and vertebrate speciation (18, 19, 26, 31). It is suspected that natural hybridization occurs between *Rana cascadae* Slater and *Rana pretiosa pretiosa* Baird and Girard, two frogs of the Pacific Northwest. Before the degree of natural hybridization can be

determined, there is need for a complete description of both the appearance and the development of the hybrid. The objective of this paper is to provide the description and to suggest a possible means of assessing natural hybridization.

Rana cascadae was described as a new species in 1939 (22, 35). Since that time its status has been disputed. It has been treated as a subspecies, Rana aurora cascadae (27), but recent evidence suggests it should be given species status (6, 22). Its range is limited to four areas: the Olympic Mountains; the Cascade Mountains of Washington, where the type specimen was found at 5,000 feet; the Cascade Mountains of Oregon at elevations of 3,000 to approximately 6,000 feet; and the Lassen Peak area of northern California (4, 6, 7, 22, 27).

Rana pretiosa pretiosa is found from southeastern Alaska and northern British Columbia, south and east to southwestern Alberta, and southward through northern Idaho, western Montana, Washington, northern and western Oregon, and extreme northern California (6, 27).

Although both frogs are found high in the Cascades, their ecology differs. Pretiosa, with its shorter legs, greater amount of webbing, and dorsad eyes is more aquatic. Cascadae, with its longer legs and less webbing is more terrestrial and inhabits mountain streams and the adjacent banks as well as shallow margins of

mountain lakes. In spite of their morphological and ecological differences, they have been found by Storm[1] and others to occupy the same lakes in some areas of the Mink Lake Basin, part of the Three Sister's Wilderness Area in the central Cascades of Oregon.

[1]Personal communication with Dr. Robert M. Storm, Department of Zoology, Oregon State University.

METHODS AND MATERIALS

The frogs were collected in September, 1964. The Rana cascadae were from Breitenbush Lake, Marion County, Oregon, Big Spring Lake, Wasco County, Oregon, and Three Creek Lake, Deschutes County, Oregon. The Rana pretiosa pretiosa were from Muskrat Lake and Little Lava Lake, both in Deschutes county Oregon. All collecting areas were above 3, 500 feet. Each area, to the collector's and author's knowledge, contained pure populations, i. e. areas where no hybridization could have occurred.

The animals were stored prior to the experiment in quart jars containing 1-1/2 inches of tap water, two or three animals per jar, and kept at 4° Centigrade. The water was changed at intervals of seven to ten days.

Ovulation was induced following the methodology of Rugh (23, 24). The method for pretiosa which gave the best results included removal of the female from 4° C to 16° C one hour before Rana pipiens pituitary injections. Four female pituitaries followed by two female pituitaries 24 hours later induced ovulation in 64 hours.

The cascadae female was also placed at 16° C one hour before pituitary injections. Three female pituitaries followed by one female pituitary 24 hours later induced ovulation in the female cascadae in 40 hours.

The males were placed in 16° C at the same time as their respective females. The testes were removed and macerated in 15 milliliters of tenth-normal Holtfreter's solution in finger bowls. Both the control and experimental eggs were fertilized in the same finger bowl. Fertilization occurred at room temperature (20° C). One and one-half hours after fertilization the eggs were flooded and removed to the experimental temperatures of 12° C and 16° C.

After first cleavage, the eggs were divided into clumps of seven or eight eggs, a maximum of ten clumps being allowed to a fingerbowl. Tenth-normal Holtfreter's solution was used as the culture medium. The eggs were counted at the time of distribution, and the percent fertility was determined on the basis of those eggs which underwent the first cleavage.

Five crosses were made. The crosses numbered 1, 3, and 4 (Table 1), each include four subdivisions. Two of the subdivisions are labeled controls and two are labeled experimental. Those marked controls are the result of intraspecific fertilization: e.g., Rana cascadae eggs fertilized by Rana cascadae sperm or Rana pretiosa pretiosa eggs fertilized by Rana pretiosa pretiosa sperm. The control crosses involving cascadae are throughout this paper indicated by C, and the control crosses involving pretiosa are indicated by P. Those subdivisions labeled experimental are the result of interspecific fertilization: e.g., Rana cascadae eggs fertilized by

Rana pretiosa pretiosa sperm, and the reciprocal, Rana pretiosa pretiosa eggs fertilized by Rana cascadae sperm. The experimental crosses are throughout this paper indicated by CP and PC. The first letter indicates the species which donated the eggs. A number following an abbreviation of a subdivision indicates the cross in which the subdivision occurs: e.g., CP-3 refers to the experimental subdivision of cross 3 (Table 1) in which cascadae eggs were fertilized by pretiosa sperm.

The crosses numbered 2 and 5 (Table 1) have only two subdivisions, one control and one experimental. This was not intended, but was the result of the females of the two species ovulating at different times.

The developing eggs were staged according to Gosner (9), whose paper involves modifications of methods developed by Shumway (25), Taylor and Kollros (28), and Limbaugh and Volpe (15).

At stage 26, the beginning of the feeding stages, the larvae were distributed to enameled rearing pans twelve by six by two inches in size, containing charcoal-filtered tap water. Six larvae were placed in each pan and raised on boiled spinach (fresh-frozen). The pans were kept at room temperature. Following completion of metamorphosis, stage 46, the young were fed vestigal-winged fruit flies.

Data involving developmental time and temperature were

collected through stage 25. After stage 25, body measurements of head and body length, body width, tail length, and tail depth were collected for each stage along with developmental time, temperature, and other descriptive characters. Measurements were made with the aid of a calibrated ocular on the binocular microscope, and a Helix dial caliper. The frogs were narcotized in "M. S. 222" (Sandoz Chemical Company, N. Y. C.) to facilitate mensuration. Photographs were taken from stage 26 through 46, and several specimens were preserved at each of the 46 stages.

Table I. Summary of five crosses to Stage 25.

# Cross	Subdivision	Abbreviation	Ancestry*	Place of Capture**	# Eggs	% Fertility	Temperature in °C.	Time (hours) to reach Stage 25
	Control	C	R. c. ♀	Breit.	141	10	16	312
			R. c. ♂	Breit.				
	Control	P	R. p. ♀	Musk.	295	6	16	322
1			R. p. ♂	Musk.				
	Experimental	PC	R. p. ♀	Musk.	361	1	16	312
			R. c. ♂	Breit.				
	Experimental	CP	R. c. ♀	Breit.	152	11	16	295
			R. p. ♂	Musk.				
	Control	C	R. c. ♀	Breit.	275	50	12	528
2			R. c. ♂	Breit.				
	Experimental	CP	R. c. ♀	Breit.	253	32	12	520
			R. p. ♂	L. Lava				
	Control	C	R. c. ♀	Creek	187	100	16	336
			R. c. ♂	Creek				
	Control	P	R. p. ♀	L. Lava	329	100	16	336
3			R. p. ♂	L. Lava				
	Experimental	PC	R. p. ♀	L. Lava	465	3	16	343
			R. c. ♂	Creek				
	Experimental	CP	R. c. ♀	Creek	225	100	16	348
			R. p. ♂	L. Lava				
	Control	C	R. c. ♀	Big Spr.	132	21	16	384
			R. c. ♂	Big Spr.				
	Control	P	R. p. ♀	L. Lava	374	98	16	384
4			R. p. ♂	L. Lava				
	Experimental	PC	R. p. ♀	L. Lava	355	-	-	-
			R. c. ♂	Big Spr.				
	Experimental	CP	R. c. ♀	Big Spr.	298	5	16	384
			R. p. ♂	L. Lava				
	Control	P	R. p. ♀	L. Lava	365	99	16	380
5			R. p. ♂	L. Lava				
	Experimental	PC	R. p. ♀	L. Lava	437	5	16	336
			R. c. ♂	Creek				

*R. c. refers to Rana cascadae, R. p. refers to Rana pretiosa pretiosa.

**The places of capture include: Breitenbush Lake, Marion county; Big Spring Lake, Wasco county; Three Creek Lake, Muskrat Lake, and Little Lava Lake, Deschutes county. All counties are in Oregon.

ANALYSIS OF LARVAL MEASUREMENTS

Limbaugh and Volpe (15) have demonstrated that the use of ratios has greater taxonomic value than absolute body measurements. Johnson (12) found that the ratio of body width/total length remained fairly constant during the stages 25 - 41 for Rana pretiosa luteiventris. Because tail depth was almost a macroscopically observed differential and it along with body width could be measured objectively, body width/tail depth ratios were chosen for statistical analysis.

Figure 1 gives the distribution of the body width/tail depth ratios for the CP - 3 hybrid at stage 28. The 60 individuals measured were randomly picked from several hundred and ran the gauntlet of early to late stage 28.

Figure 2 plots several means from samples of body width/tail depth ratios at various stages. It clearly shows that the hybrid is intermediate to the two parent species, but it does not give the significance of separation.

Figure 3 uses the method outlined by Hubbs and Hubbs (11). It demonstrates the significance of the differential between the hybrid and the parent species. If the gap between the solid dark bar exceeds ten percent of the length of the shorter, the significance of the difference between the populations may be regarded as demonstrated

beyond any reasonable doubt (if we assume the samples to be representative) (11, p. 41). The method of measuring mentioned in connection with Figure 1 is assumed to yield representative samples. Therefore Figure 3 is a valid demonstration of the intermediate and significant difference possessed by the hybrid and expressed by the body width/tail depth ratio.

Table 2 employs the student t-test (14) to again demonstrate the significance of the hybrid differential at selected stages. In addition it supports the consistancy of body width/tail depth ratios within the same genotype through a period of time by comparing early and later stages.

Figure 4 gives the developmental time for the CP - 3 hybrid from stage 25 through stage 46. The developmental trend for Rana cascadae and Rana pretiosa pretiosa was determined from Storm's data[2] and superimposed upon the graph for comparative purposes.

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[2]Unpublished material of Dr. Robert M. Storm, Department of Zoology, Oregon State University.

Table 2. Student t-test comparing body width/tail depth ratios at selected stages.

Genotype	Stage	vs. Genotype	Stage	Degrees of Freedom	Value of t	Significance at 95 Percent
CP	28	C	28	92	11.1498	Different
CP	28	P	28	91	11.0140	Different
P	28	P	39	42	1.5674	Same
CP	28	CP	39	91	0.4486	Same

Figure 1. Distribution of body width/tail depth ratios for CP - 3 at stage 28. N refers to the number of individuals measured; the long horizontal bar represents the range; the short vertical bar is the arithmetic mean; the solid horizontal rectangle represents four standard errors, two on either side of the mean; one half of the solid rectangle plus the hollow rectangle on either side represents one standard deviation.

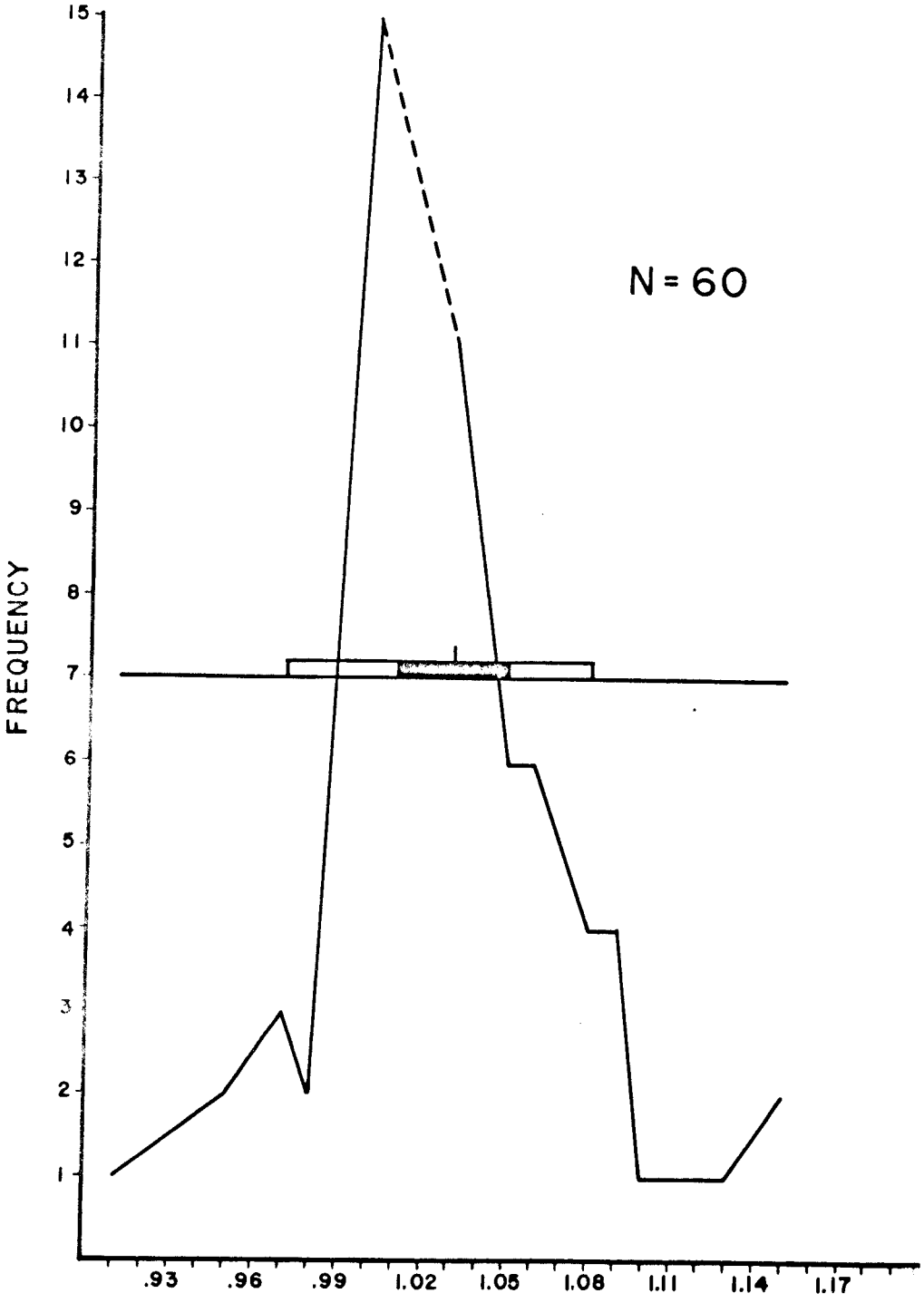

N = 60
FREQUENCY
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
.93
.96
.99
1.02
1.05
1.08
1.11
1.14
1.17
RATIOS OF BODY WIDTH TO TAIL DEPTH FOR CP-28

Figure 2. Mean ratios of hybrid and parental species at selected stages. The C line connects the mean ratios of Rana cascadae; the CP line connects the mean ratios of the hybrid; the P line connects the mean ratios of Rana pretiosa pretiosa; the dotted lines connect means from non-consecutive stages.


MEAN RATIOS OF BODY WIDTH TO TAIL DEPTH
1.30
1.28
1.26
1.24
1.22
1.20
1.18
1.16
1.14
1.12
1.10
1.08
1.06
1.04
1.02
1.00
.98
.96
.94
.92
.90
.88
.86
.84
.82
C
CP
P
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
STAGES OF DEVELOPMENT

Figure 3. A graphical analysis and comparison of a series of samples of body width/tail depth ratios for both the hybrid and parental species. The long horizontal bar represents the range; the short vertical bar is the arithmetic mean; the solid horizontal rectangle represents four standard errors, two on either side of the mean; one-half of the solid rectangle plus the hollow rectangle on either side represents one standard deviation; C represents Rana cascadae; CP the hybrid; P, Rana pretiosa pretiosa; the number following the capital letters refer to a particular stage.

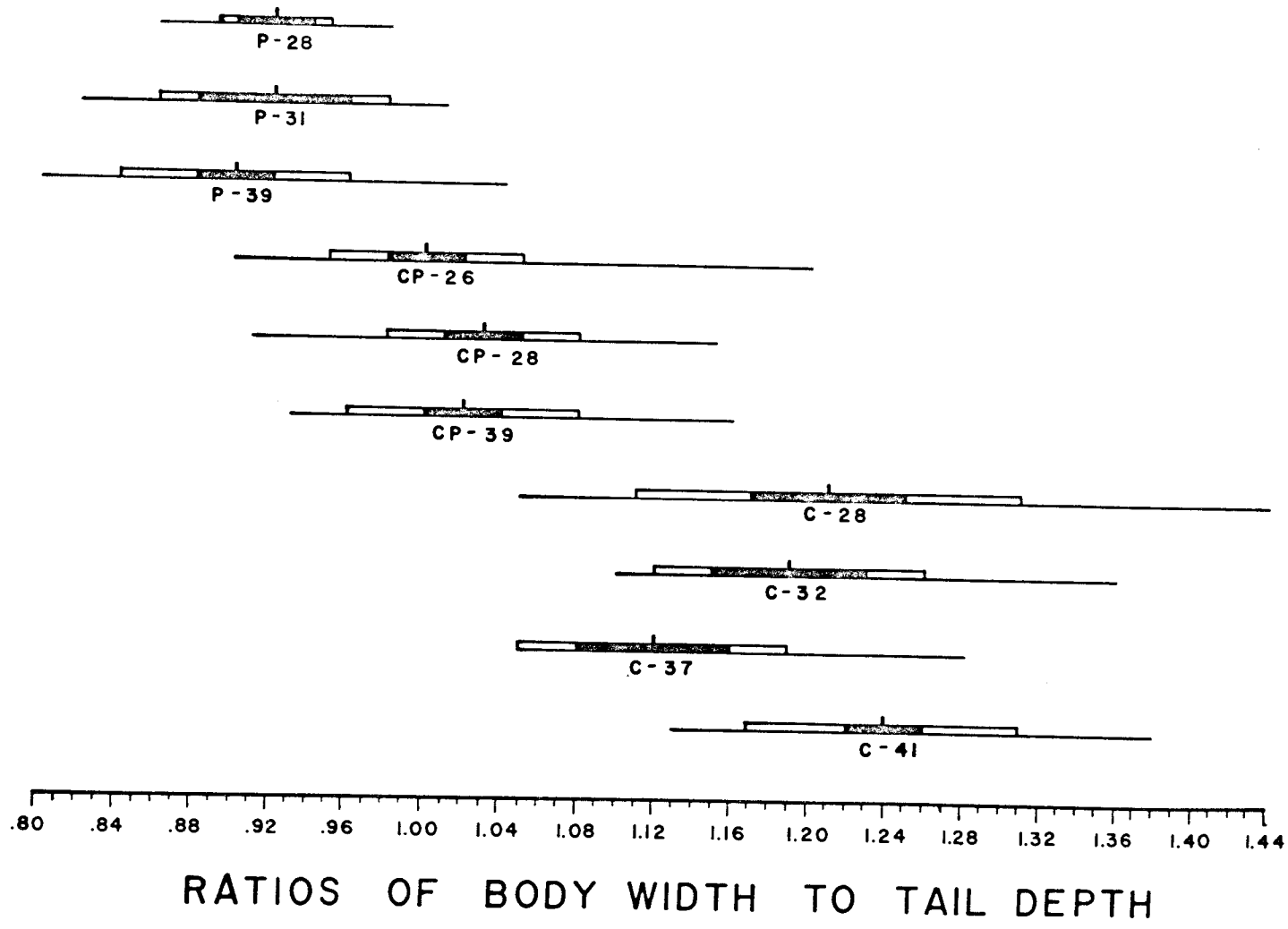
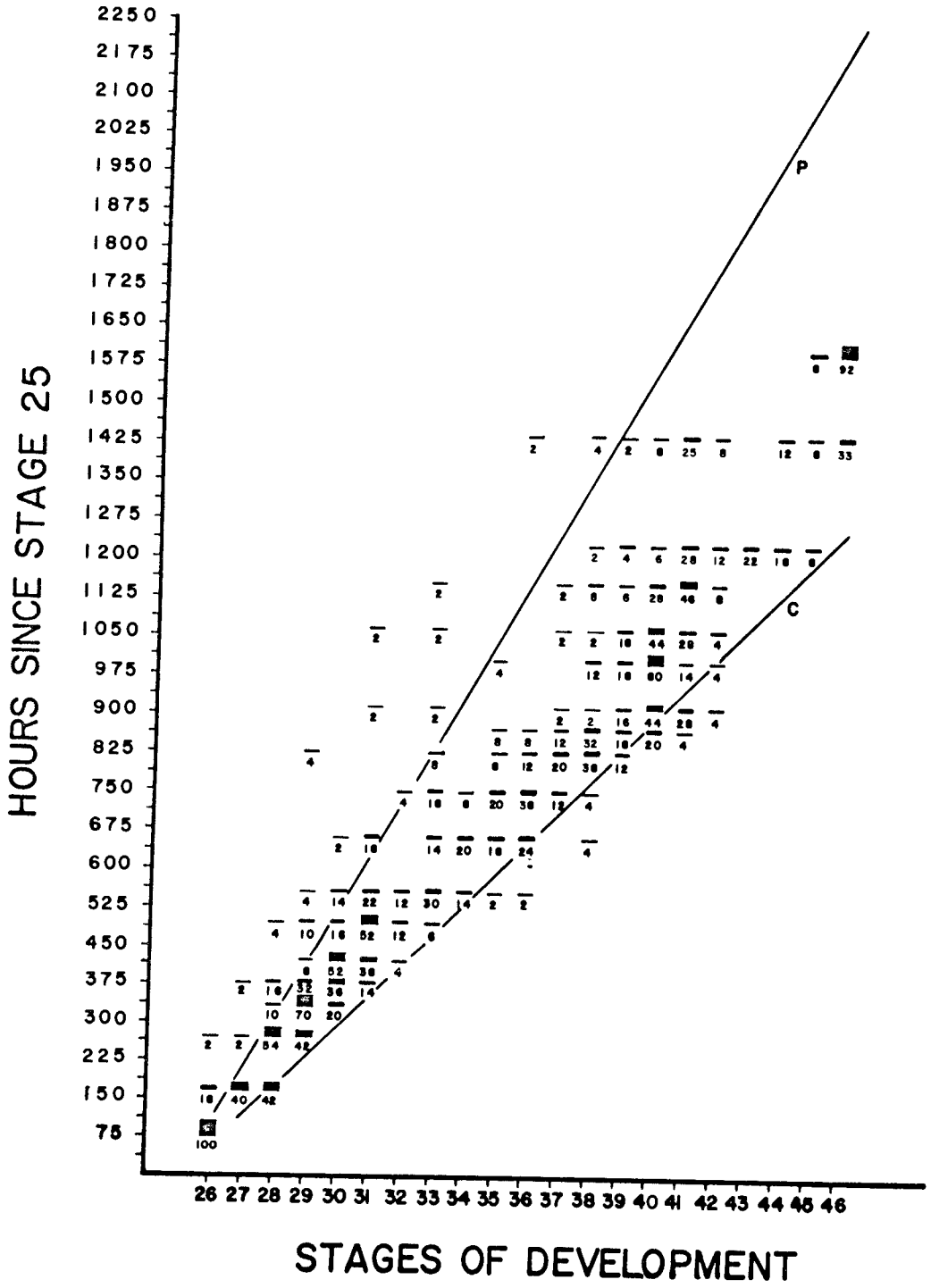

P - 28
P - 31
P - 39
CP - 26
CP - 28
CP - 39
C - 28
C - 32
C - 37
C - 41
.80
.84
.88
.92
.96
1.00
1.04
1.08
1.12
1.16
1.20
1.24
1.28
1.32
1.36
1.40
1.44
RATIOS OF BODY WIDTH TO TAIL DEPTH

Figure 4. Bar graph expressing developmental time from stage 25 through 46 of the hybrid at 19° C. The number beneath each bar represents the percentage of larvae that exhibit the characteristics of the stages at the hour indicated; the oblique line marked P gives the developmental trend for Rana pretiosa pretiosa; the oblique line marked C gives the developmental trend for Rana cascadae.


HOURS SINCE STAGE 25
2250
2175
2100
2025
1950
1875
1800
1725
1650
1575
1500
1425
1350
1275
1200
1125
1050
975
900
825
750
675
600
525
450
375
300
225
150
75
P
C
26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46
STAGES OF DEVELOPMENT

LARVAL MOUTH PART COMPARISONS

The mouth parts for Rana pretiosa pretiosa were differentiated into all of the components by stage 28. The horny beak was black, peppered with tiny iridiphores. There were two rows of chitinized teeth in the upper labium and three rows in the lower labium; this is represented by the formula 2/3. The first upper row was complete; the second row lacked the medial portion. The first lower row lacked the medial portion; the second lower row was longer than the first; the third lower row was complete, and is the shortest, being about the same length as the width of the upper beak. The labial papillae were confined to the lower labium. In one individual, reabsorption of mouth parts started at stage 38. By stage 42 the beak had disappeared in all of the individuals; a few lateral papillae were all that remained of the larval mouth components. Stage 34 is diagramed in Figure 5.

By stage 29 the hybrid had all of its mouth components. It maintained them through stage 41. The tooth formula was 2/4. The numerator is the same for Rana pretiosa pretiosa and the denominator is the same for Rana cascadae. The tooth rows were a little wider in proportion to the width of the beak in comparison with that of the parent species. One of the reciprocal hybrids had the same 2/4 formula. Variation was noted in the completeness of the rows.

In most cases row one of the upper labium was complete and row two lacked the medial portion. In the lower labium row one lacked the medial portion and rows two, three, and four were complete. In one individual all of the tooth rows in both labia lacked the medial portion. In another individual rows one and two of the lower labium lacked the medial portion. No individual was found in stages 28 through 41 which did not have a 2/4 formula. Stage 34 is diagramed in Figure 5.

The mouth components of Rana cascadae were slower in development. At stage 27 the formula was 2/3 as in Rana pretiosa pretiosa; at stage 30 the formula was 2/4 as in the hybrid; and at stage 33 the formula was 3/4. Except for one individual with a formula of 2/3, the formula 3/4 was maintained through stage 40. This demonstrates variation which may occur in the components of the mouth parts and the need for expressing the formula at a particular stage. It also points out the necessity of studying several individuals at any particular stage to determine the major formula. Stage 34 is diagramed in Figure 5.

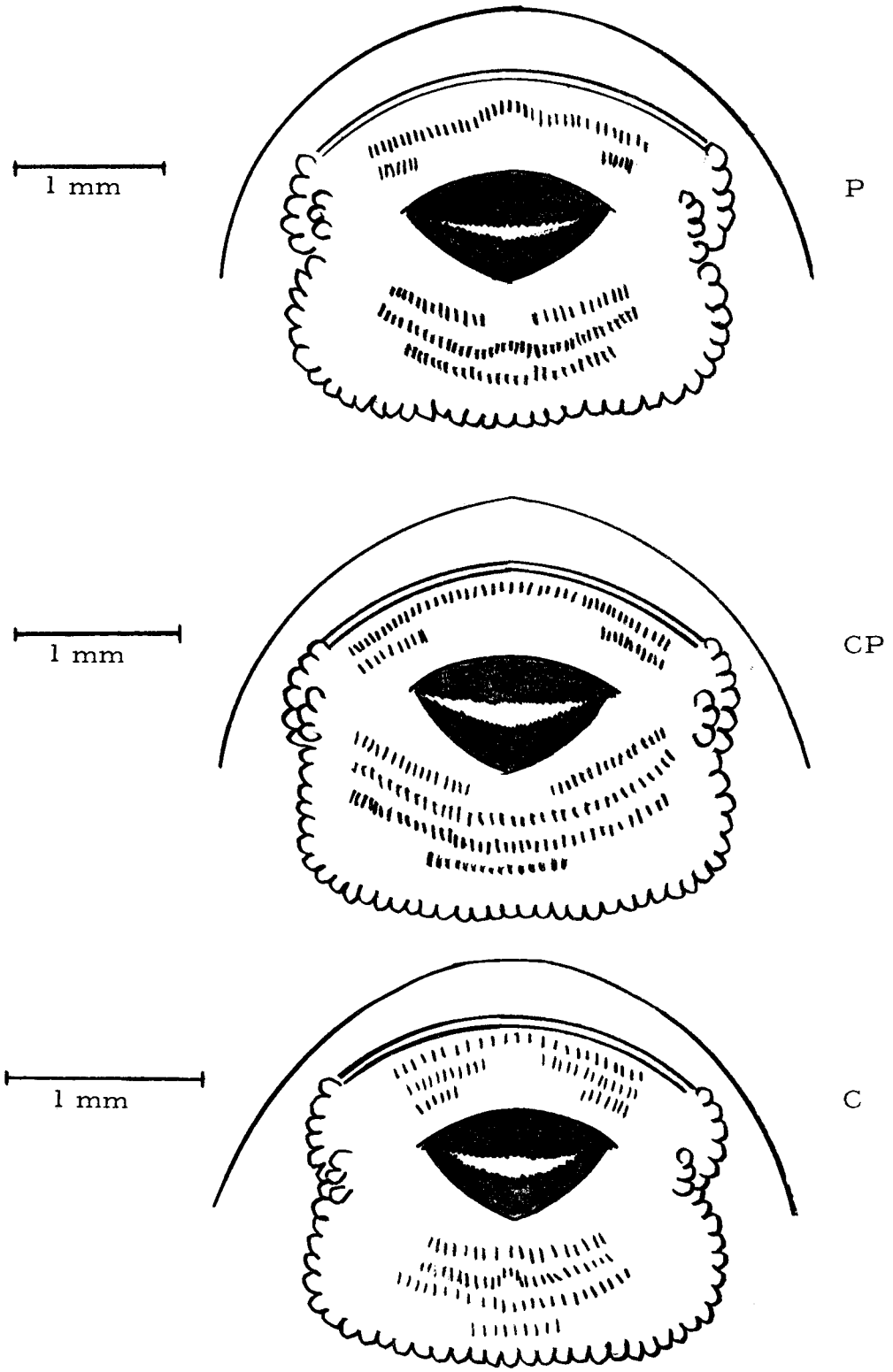


Figure 5. Hybrid and parental species mouth parts at stage 34.

EXTERNAL DESCRIPTIONS OF HYBRIDS AT SELECTED EMBRYONIC STAGES

Embryonic stages refer to the pre-feeding stages, or the first 25 stages (9). At stage 13 the neural plate was a "beanie-cap," differentiated from the rest of the sphere by a stretched gap. At stage 15, the embryo exhibited a rough-skinned appearance which it maintained until stage 24. Rotation of the embryo (stage 15) was recognized by the use of a two-second time exposure. At stage 18, the embryo showed more arching than Rana pipiens.

Hatching occurred during stages 19 through 21. At the time of hatching the entire embryo was a sooty brown. Length at this time ranged from 7.9 mm to 9.7 mm.

The heart beat (stage 19) was difficult to see but was recognized by the following procedure. A beam of light was directed anterior to posterior over the ventrum of the animal casting a shadow over the heart region. Movement of this shadow indicated the beating heart (12).

Gill circulation (stage 20) was detected on the average 44 hours after the heart beat by means of side and back lighting. Since gill pigmentation was heavy, gill circulation may have begun some time before it was detected.

Size of gills varied considerably. Generally there were two main trunks from which palmately and oppositely arranged branches

occurred. A smaller, unbranched trunk occurred posterior to the larger two. Embryos reared in crowded conditions tended to have larger and more branched gills. The degree of variation present indicated that gill size and shape would not be useful as species-characteristic traits.

The same detection problem that occurred with gill circulation occurred with tailfin circulation, and the initial phase may or may not have been detected. Tailfin circulation was first noticed at the dorsal body-tailfin junction.

Other workers have found the mouth-open characteristic to coincide with the transparent cornea characteristic (9, 12, 25). The two characteristics are supposed to label stage 21. However, in the case of the hybrid, cornea transparency occurred anywhere between stage 21 and stage 24.

At stage 23 patternless melanophores and lipophores were present. At stages 24 and 25 peppered iridophores were present. Patterning of iridophores first appeared at the iris of the eye and in two parallel bands at the body-tail musculature junction.

At stage 25 the body was peanut-shaped from above and slightly dorso-ventrally compressed. The spiracle was sinistral and located less than two-thirds the body length from the anterior end. The anus was dextral. Coloration was sooty brown, sprinkled with iridophores, melanophores and lipophores. Peppered melanophores

tended to enhance the somite pattern of the tail musculature. The tail fin was transparent grey. The posterior margin of the eye coincided with the posterior margin of the anterior third of the body. The tail length was less than twice the body length and the tail musculature did not reach the tip of the tail. At the point of greatest depth of the tail fin the musculature was dorso-ventrally centered and was equal to one-third the total depth of the fin. The average length of 60 individuals at stage 26 was 17.2 mm with a range from 14.3 - 20.1 mm.

The embryonic stages which were the easiest to identify and therefore the most objective were: 3-6, 10-18, 22, and 24-25. In comparative studies the author would suggest that greater value be placed on data from these stages.

EXTERNAL DESCRIPTIONS OF HYBRIDS AT SELECTED LARVAL STAGES

Larval stages include those numbered 26 through 46. In stage 30 the external nares were anterior to the midpoint between the tip of the snout and a line passed vertically through the center of the eye. The tail was approximately 1.4 times the body length. The tail musculature was still approximately one-third the total fin depth but was now located slightly below center. The greatest depth was reached in the anterior half of the tailfin giving it a more lanceolate than elliptic shape. The metallic iridophores had increased in number and coalesced into irregularly shaped clumps of about 0.9 mm diameter. There were as many as 30 in a clump. The clumps were numerous and randomly spread over the dorsum and lateral parts of the body with a gradual thinning in numbers anteriorly. The clumps tended to show some degree of parallelism over the posterior half of the dorsum with the lines running anterior-posterior in direction. On the venter the iridophores formed a solid shield over the coiled intestine. They thinned out and ended 1 mm posterior to the lower labium. Lipophores were present, but the color appeared to be dispersed rather than clumped. Melanophores still appeared peppered on and were more numerous on the upper half of the tail fin. Filamentous melanophores were present on the dorsal edge of the legs, giving a hirsute appearance. Amount of color increased during

the stages following 30 and was most easily observed on the dorsal surface of the leg. All chromatophores worked toward a counter-shading effect; darker on the dorsum and lighter on the venter. Chromatophophores were found both on the surface and in the deeper tissues. The pattern on the surface appeared independent of the pattern in the deeper tissues. The average length of the individual at stage 30 was 35.4 mm with a range from 27.7 to 41.4 mm.

In stage 38 the proportions were still similar to those of stage 30. The most noticeable change was in coloration. The pigmentation was less blotchy and metallic and more even. Iridophores lined the muciferous crypts and melanophores outlined the tail blood vessels.

In stage 41 alternate green and black banding was apparent on the dorsum and rear legs, extending almost to the tip of the toes. The completeness and clarity of the bands varied. Three to five bands were present on the thigh and four to five on the shank. On the dorsum, spotting was apparent posterior to the eyes. The spots were formed by melanophores and had a light area in the center. They were located within a rectangle bounded by a line through the eyes, the lateral folds, and the tail-body junction. Smaller, warty spots were present on the dorsal surface of the thigh and shank. A mask was beginning to be formed around the external nares.

EXTERNAL DESCRIPTION OF THE YOUNG HYBRID FROG

The dorsum showed heavy spotting with the larger spots more posterior. The spots had blurred edges; a few had light centers. The dorsolateral folds were coppery red. Fine golden to coppery guanophores were present over the back, on the exposed leg surfaces, and almost solidly on the dorsal head surface. There was a black mask through the external nares, and a white lip line which extended to the forelegs. The dorsad surface showed incomplete barring on the forelimbs, four complete bars on the femur, four complete bars on the tibia, and four complete bars on the tarsus. The overall color was brownish-green.

On the ventral surface, the throat was mottled with pale yellow-red blotches. A few small blotches were found on the abdomen, and mottling also occurred in the groin. The anterior venter of the thigh was yellowish with a tan suffsion. The posterior ventral metatarsal area was orange-red, and the anterior part was yellowish. Yellow extended onto the foot but the sides of the toes were reddish. The ground color of the ventral surface was cream.

Webbing was like that in Rana pretiosa pretiosa. The eyes were not as dorsally located as in Rana pretiosa pretiosa. There was variation in the amount of dorsal spotting and in the clearness of

the abdomen. Size at metamorphosis averaged 21.9 mm. The range was from 18.9 mm to 24.3 mm.

DISCUSSION

In the resume of crosses, Table 1, one notices the marked decrease of fertilization of Rana pretiosa pretiosa eggs by Rana cascadae sperm. In cross 3, fertilization was three percent successful as opposed to the reciprocal's 100 percent success. This phenomenon is indicated by all five of the crosses. This lack of success may be due to shortcomings in technique or may result from a primary incompatibility between pretiosa eggs and cascadae sperm. Reciprocal differences have been shown by Volpe (30) between Bufo valliceps and B. fowleri, but the differences were ascertained at gastrulation and not at fertilization. The author has one male hybrid from the PC cross which is very much alive and over a year old at this writing.

The intermediacy of characters including: body width/tail depth ratios, developmental time, larval mouth parts, and to a lesser degree external pigmentation, expressed by the hybrid in comparison with the parent species argue for a 2N hybrid. Both paternal and maternal chromosomes determine the characteristics of the viable hybrid. Interspecific crosses among the bufonids and hylids also yielded hybrids which blended characters of the paternal species (13, 16, 17, 29, 30, 32, 33).

Dumas (6) made an experimental cross involving the same

species as the author. His hybrids showed no reciprocal differences at fertilization and none reached gill circulation. From his results he concluded that Rana pretiosa and Rana cascadae were identifiable species. His other studies, electrophoretic and protein precipitate, did however indicate a closer relationship of Rana cascadae to Rana pretiosa pretiosa than of Rana cascadae to Rana aurora aurora. The author's data would confirm this close relationship if one assumes viable hybrids indicate a close relationship. Dumas found intra-specific variation of the β globulin in both species. His Idaho pretiosa had three β globulin peaks, while his Oregon cascadae had only two. He found two β globulin peaks in pretiosa from south central Washington. The author's pretiosa should be comparable to the latter, and the crosses in this paper should then have involved frogs with similar β globulin peaks. Perhaps there is greater compatibility between Oregon pretiosa and Oregon cascadae than between Idaho pretiosa and Oregon cascadae.

The body width/tail depth ratios proved to be the best single characteristic for separating the hybrid from either of the parent species (Figure 3 and Table 2). Limbaugh and Volpe (15) have shown that ratios may remain constant for stages 26 through 41. The student t-tests, Table 2, support this observation, where a comparison of stages 28 to 38 of individuals with the same parents proved them to be of the same population.

Variation in developmental time is evident (Figure 4). This differential would change the hybrids' chance for survival. It can be assumed that in a natural population individuals of the hybrid in stage 46 would be searching for prey after cascadae had graduated to larger prey items and before pretiosa was after any. The new niche occupancy could lessen competition with the parent species. A reverse argument could be given: i.e., that the prey feeding stage of the hybrid may arrive when prey items are few in number. Although variation in developmental time indicates a survival differential, it does not offer an easy means of determining the ancestry of an unknown population. One would need knowledge of when eggs from the population in question were fertilized and a means of comparing them through a period of time to a known control.

The generalized tooth formulae for stage 34 of 2/3 for Rana pretiosa pretiosa, 3/4 for Rana cascadae, and 2/4 for the hybrid were given for the mouth parts. Cascadae larva have been found with a 2/4 formula (35, p. 341). Laboratory raised cascadae had a 2/4 formula during stages 30-34. The 3/4 formula appeared during stage 34. Wild cascadae larvae at stage 39, collected from Three Creek Lake, Oregon had formulae of 3/3 and 3/4. Wright and Wright (35, p. 524) give 3/3 and 2/3 as formulae for Rana pretiosa pretiosa. All of the pretiosa controls had a formula of 2/3 during stages 28-38. Wild pretiosa larvae at stages 28-41, collected from Muskrat Lake,

Oregon had a 2/3 formula. The hybrid had full components of mouth parts during stages 28-41. The literature asserts that there is a considerable amount of variation which can occur with anuran larval mouth parts (5, 10, 20, 21, 35). The author is in full agreement with this. Before the value of the "generalized formula" can be ascertained as a tool for separating hybrids in nature from the parental species, further collections of natural controls must be made and their tooth formulae at particular stages analyzed. Usefulness of this character is indicated during stages 35-38 by the laboratory data.

The major tool usable in the field for studying natural hybridization is the body width/tail depth ratio. This can be used during the summer months when the mountain lakes are accessible and larvae are present. If one collects the larvae, separates them into various stages, and statistically treats the body width/tail depth ratios, the resulting graph, in the same format as Figure 3, will indicate the amount of gene exchange. It will also be necessary to collect larvae from known areas of isolated controls and to check their ratios with the graph.

If F_1 hybrids are sterile but the controls continue to hybridize as a result of faulty isolating mechanisms, one would expect three distinct populations as shown in Figure 3. The same results would be obtained if F_1 hybrids are fertile among themselves and

maintain a separate population.

If F_1 hybrids are fertile and capable of producing offspring with the parental species, one would expect a spectrum of ratios throughout the entire range, and no distinct populations. Skewness toward either end would indicate either a tendency toward successful breeding with one parent or a greater abundance of one species over the other, a phenomenon common where hybridization occurs (18, 26).

Finally, if it appears from the field study that hybridization does occur, adults from the area can be collected, artificially crossed in the laboratory, and the known progeny characterized by time of development, body ratios, tooth development, and general appearance. The total picture will then give information as to the amount and direction of natural hybridization.

SUMMARY AND CONCLUSIONS

Experimental crosses between Rana pretiosa pretiosa Baird and Girard and Rana cascadae Slater, resulted in viable hybrids. Reciprocal differences at the time of fertilization are indicated. A high level of success in producing viable hybrids can be obtained if Rana pretiosa pretiosa is the female of the parent species. The hybrid is characteristically intermediate to the parent species.

The hybrid is quantitatively intermediate to the parent species in the characteristics of larval body width/tail depth ratios, developmental time, and mouth parts.

Statistically treated larval body width/tail depth ratios of natural populations can be used to determine the amount and direction of natural hybridization between the species.

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