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From the Amazonriver to the Amazon molly and back again

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CHARACTER DEVELOPMENT AND EVOLUTIONARY MECHANISMS IN *POECILIA*

"As a result of our recent studies on [poeciliid] fishes, including breeding experiments as well as systematic and variational studies of natural forms throughout their range, weight is constantly being added to the theory that speciation is no aimless wandering of genes through the organic world, but rather an orderly adjustment under the rigid control of the environment." [Hubbs, 1940]

Abstract
To investigate the present infrageneric classification of *Poecilia*, or rather the lack of one (cf. Breden et al., 1999), an understanding is necessary of ‘how the characters came to be’. This chapter includes several mechanisms that influence character development in *Poecilia*, divided into three major groups: character development caused by ecological, abiotic factors, i.e., ecomorphological factors, character development caused by ecological, biotic factors, i.e., character displacement, and speciation caused by hybridization. These mechanisms are illustrated by re-interpreted examples from literature and my own investigations.

Knowledge of character development can then be included in further studies on supraspecific classifications.

Introduction
The species of *Poecilia* are small to medium large species of fish, with adult specimens ranging from 10 mm SL to over 100 mm SL. The dorsal fin is often positioned midway between head and tail, as does the anal fin in the female. In males, the anal fin is positioned more anteriorly and is modified into an intromittent organ, the gonopodium. Meristic counts in *Poecilia* vary within limits: most species have 14, 16 or 18 scales around the caudal peduncle, between 26 to 30 scales in a lateral series, eight or nine anal fin rays. The number of dorsal fin rays, however, range from six to eighteen in this genus.

Based on dorsal fin ray number two Central American species groups are recognized in *Poecilia*, viz., the *P. latipinna* species group (modally more than 12 dorsal fin rays) and the *P. sphenops* species group (modally less than 11 dorsal fin rays). Three species appear to have originated through hybridization of members of these two species groups (Section 7.3). Taxonomic difficulties concerning the *P. sphenops* complex in the past were highlighted in the “introduction to this thesis”. To gain insight in this problem, I have subdivided the *P. sphenops* species group in the present chapter. Six widely distributed species, viz., *P. sphenops*, *P. mexicana*, *P. gillii*, *P. butleri*, *P. nelsoni* and *P. marcellinoi* (see Chapter 11), are hereafter defined as the *P. sphenops* complex (Figure 1), separating them from the remaining seven species of the *P. sphenops* species group that have a more restricted ranges, viz. *P. thermalis*, *P. pallida*, *P. sulphuraria*, *P. chica*, *P. catemaconis*, *P. teresae*, and *P. boesemani*. 
In South America there are less species (14 vs. 18 in Central America) but more species groups, viz., the *P. vivipara* species group (four species), the guppy (one species: *P. reticulata*), the *P. caucana* species group (three species) and the *P. parae* species group (five species).

Three species, viz., *P. elegans*, *P. dominicensis* and *P. hispaniola*, occur in Hispaniola.

The overlap of characters between species, and variation within species (Figure 1), made taxonomic differentiation above species group level difficult. The array of characters examined previously, including relative fin positions, dental characters, intestinal lengths, fin ray counts, gonopodial structures, gonopodial suspensorium, and molecular data have not resulted in a stable taxonomy or a resolved phylogeny (Rodriguez, 1997; Breden et al., 1999). This chapter investigates character expression in *Poecilia* and examines the usefulness of a number of features. In understanding the origin of the characters, their importance will come to light. The mechanisms that shape characters in *Poecilia* include ecomorphological character developments (Section 7.1), character displacement (Section 7.2), generally considered a "major evolutionary mechanism (Futuyma, 1979)" and hybridization (Section 7.3), already proven for *P. formosa* and herein postulated for two other species.

This chapter does not supply an account of the species, the species are catalogued in Chapter 11.

7.1 Ecomorphological character development

In a series of papers, Hubbs (1922, 1924, 1926b) observed a correlation between environmental agents and character development in fish. Fish developing in a saline or in a cold environment have enhanced meristic and somatic development, i.e., have more fin rays and vertebrae, and obtain a larger size at maturity. Originally defined by Hubbs (1922, 1924, 1926b), this is a phenomenon linked to differences within a species, and supposedly direct speciation, i.e., eventually lead to differences between species (Hubbs, 1926b).

Hubbs (1940) noted a remarkable exception for the Central American poeciliids in this trend. Towards the north, and also toward the uplands, a decrease in meristic characters was observed. This seemed to contradict the temperature-element in character development, which predicts an increase in the number of fin rays and scale counts towards the cooler north and the cooler upland environments.

Ecomorphological characteristics in *Poecilia*

The ecomorphological mechanism of Hubbs was confirmed in field studies for salinity and body sizes of *P. vandepolli* (cf. Feltkamp and Kristensen, 1969) and *P. gillii* (cf. Meek and Hildebrand, 1916). *Poecilia vandepolli* is a polymorphic species of the subgenus *Poecilia*, occurring on the Leeward group of the Lesser Antilles (Aruba, Curaçao, and Bonaire), and on the island of St. Maarten (= St. Martin, on the French side of the island) of the Windward group (Chapters 1 & 5). Feltkamp and Kristensen (1969) published a study on morphological variations in *P. vandepolli*. They conducted a superficial study on the South American mollies
and concluded that all mollies of the Venezuelan coast, as well as on the Dutch Antilles were conspecific, \textit{viz.}, \textit{P. vandepolli}. Populations of mainland fishes had irregular spots, distinguishing them from the island populations. In more detailed studies (Chapters 1 & 5) several prominent differences were found, justifying a separate specific status for the mainland populations. Moreover, specimens of \textit{P. vandepolli} from Aruba also were encountered with irregular spots, hitherto only known from Venezuela (cf. Feltkamp and Kristensen, 1969). Spotted specimens are also known from other species of \textit{Poecilia}, e.g., \textit{P. latipinna} (Figure 2), \textit{P. mexicana} (Figure 3), \textit{P. sphenops}, \textit{P. orri} and most notably in \textit{P. latipunctata}. The males of \textit{P. vandepolli} are polychromatic. The body can be grayish brown, like the females, it can be yellowish with blue, or has an orange throat with a black margined orange dorsal. In several populations the males have an obvious black humeral blotch, positioned behind the pectoral fins (Chapter 5).

![Figure 1](image1.png)

**A.**

Figure 1. Habitus of the species of the polymorphic \textit{Poecilia sphenops} complex (drawing of R. Wildekamp). All species contain slender specimens (A) as well as higher specimens (B). In addition, all species are polychromic, exhibiting a wide array of body and fin colorations.

B.

The morphological differences observed in nature by Feltkamp and Kristensen (1969) were superficially related to the salinity of the habitat. \textit{Poecilia vandepolli} occurs in fresh water, as well as in salt water and supersaline water. The specimens collected from the sea water
environments were larger than those caught in fresh or supersaline water. These observations confirmed similar results for Panamanian populations of *P. gillii* (cf. Meek and Hildebrand, 1916). Feltkamp and Kristensen (1969) found that seawater specimens, as opposed to supersaline specimens, grew faster, and had more pectoral fin rays and more caudal fin rays. As opposed to fresh water specimens, individuals collected from seawater grew faster, had more pectoral fin rays and more caudal fin rays. In addition, the females had deeper caudal peduncles and fewer scales in the lateral series. The relatively shorter head and smaller eyes of these individuals were attributed to the increased somatic growth. In the laboratory, some influence of salinity was observed on the fin ray averages and scales in a lateral series, although no direct correlations were found. They concluded a firm influence for salinity on growth, although other factors were found as well with some impact on growth, e.g., the fishes grew better in running water than in stagnant water. There was no apparent significant genetic component for the factors examined. Their results explained the prominent ecological influence and the lack of a significant genetic component linked with the migrational habits of the species. After heavy rainfall, coastal populations tend to migrate upstream. This causes a thorough mixing of populations, counteracting segregation of genetic entities.

Figure 2. Melanistic specimen of *Poecilia latipinna* (drawing of R. Wildekamp)

Ecotype (or subspecies) formation in *P. mexicana* I

The recognition of geographically separated subpopulations or subspecies in widely distributed species is herein fully explained by ecomorphological differentiation. For example, in *P. mexicana* "mexicana" and *P. mexicana* "limantouri", an ecomorphological differentiation in body shape and pigmentation patterns is present in upstream populations versus downstream populations (cf. Menzel and Darnell, 1973). This differentiation, which in *P. vandepolli* was counteracted by structural migrational behavior (cf. Feltkamp and Kristensen, 1969), is caused in *P. mexicana* by ecological changes over a great distance. However, as pointed out above, the observed gradient of character development seemingly contradicts the mechanism proposed by Hubbs (1922, 1924, 1926b). The populations that occur in the north, which simultaneously occur more inland and thus upland, differ from the
downstream populations in having a smaller body size and lower values to meristic characteristics, although they exist in cooler circumstances. One explanation for this anomaly is that the inland habitats are not only cooler, but also less saline. Saline environments have the same effect as cooler conditions, i.e., specimens grow larger and have a larger number of fin rays and scale counts.

Figure 3. Melanistic specimen of *Poecilia mexicana* (drawing R. Wildekamp)

For the widely spread *P. mexicana*, sheer distance results in genetical stable populations, i.e., ecomorphs. This ecological expression is often translated into the taxonomical expression of subspecies. Subspecies formation is aided by habitat preference of individual specimens, e.g., slender specimens avoiding competition of the larger specimens by migrating upstream, and larger specimens forced to drift down by the stronger upstream currents, thus separating the population in slender upstream individuals and larger downstream individuals. Any incipient genetic component concerning size differentiation is thus reinforced, favoring the development of larger specimens downstream and the more slender types upstream. Consequently, it is unnecessary to explain the occurrence of two types of *P. mexicana* populations involving geological events that separate and re-unite populations (cf. Menzel and Darnell, 1973).

Ecotype (or subspecies) formation in *P. mexicana* II

A case of distinct intraspecific differentiation is recorded for so-called normal mollies and the "cave molly" (Gordon and Rosen, 1962). The fish, identified at the time as populations of *Molliesenia sphenops*, were discovered in 1944 in a stream in the Mexican State of Tabasco, on the northern sides of the Chiapan Sierra Madre. The physical conditions of the area were described in detail and Gordon and Rosen added a map, so the abiological conditions of the habitat are fully appreciated. The stream is situated well above any connected river and it seems likely that this area has been isolated for a considerable time. *Poecilia mexicana* is probably the only species of fish inhabiting the stream that is partly situated in a cave. Many small and pale specimens from the cave-environment were collected, together with
conspecific fish from outside the cave. A clinal gradient in morphometric characters is noted in the normal *P. sphenops*-like fishes and a fully adapted cave morph. The cave form is described as follows (Gordon and Rosen, 1962): "A slender, almost colorless form of *Poecilia sphenops* with a thick-lipped terminal mouth and a small eye, the edges of which are obscured by delicate fleshy folds of circumorbital tissue. Gape of retracted mouth horizontal rather than oblique. Dorsum in front of dorsal fin origin moderately to strongly arched downward toward the terminal mouth. Papilla-like swellings bordering the anterior margin of the open posterior remnant of the infraorbital canal (representing head pores 4b, 5 and 6a when the canal is closed over). Adult female with a large fleshy genital pad that envelops the bases of anal rays 1 and 2." Extra tables were included with morphometric and meristic data and suggested quite strongly that the cave dweller preferred the bottom reaches of its surrounding, the subpopulation from outside the cave had in the laboratory a preference for the middle and the upper levels of their habitat.

In this case, it is obvious that the physical conditions of the cave environment versus the normal river environment is responsible for the observed gradient in morphology between the extremes. Rosen and Gordon concluded, as the case may be uncritically, that the variation in characters between the river inhabiting fish and the specialized cave mollies required a period of isolation between the two forms. Furthermore, they stated: "The fact that the pattern of morphological variation persists is circumstantial evidence for the existence of an as yet unknown feeder population of a true cavernicolous form upstream from the known cave." The possibility of an even more adapted form kept them from applying a subspecific status for the cave mollies. Based on the assumption that the morphological differentiation results from a time of separation, the authors speculated as to how long two populations can be separated (and subsequently differentiate morphologically) without becoming genetically incompatible.

An alternative which Rosen and Gordon did not consider involves an environmental or epigenetic control of character expression. For example, in *Astyanax mexicanus*, a cyprinid, also a cave form conspecific to surface populations is known. In this species "... striking morphological differentiation (is) not accompanied by large allozyme differentiation, between the isolated cave and surface populations ..." Although these two surface and cave populations have indeed been separated, no significant genetic diversification has occurred. This would indicate that large morphological differences do not have to arise from genetic diversification, but can also develop when the environmental gradient is large enough. Also in the genus *Cyprinodon*, an egg-laying toothcarp, "... marked habitat differences ... may cause striking divergence in some adaptive anatomical or physiological trait, without altering a major portion of the genome (Avise, 1975)." Specimens of the genus *Cyprinodon* are found in separated habitats in which they exhibit large interspecific morphological differentiation with little genetic variation. The species in this genus are capable of interbreeding under laboratory conditions and have fully fertile F1 hybrid offspring.
Character development in *P. reticulata* caused by predation

Guppies are fishes with an incredible polychromatic variety. Even with the overwhelmingly detailed description of the genetics and evolutionary properties of the colors in guppies, the general significance of this feature is ill-understood (Houde, 1997). Guppy color patterns have been investigated since the nineteen-twenties (Winge, 1922), and many patterns of black, red, orange, green and yellow spots have been described. The amount of “red” on the body was found to be of special interest in evolutionary studies focusing on female preference in color inheritance (Endler, 1980; Houde, 1997). This sexually selected trait was linked to the presence of predators and parasites, indicating male fitness. The amount of “black” was considered as mere accentuating the red spots, i.e., acting neutrally in the evolutionary process. Winge (1922) already noticed that not all patterns are stable, e.g., in some cases a red spot might or might not occur. In the cases that “a little red side-spot occurs more or less constantly on the front of the breast,” he noted that: “it is not impossible that the autosomes may contain important factors” causing this variation (Winge, 1922: 150).

An unexplained feature of the color patterns related to predation is the presence of these patterns on the Y-chromosome or the X-chromosome. It appears that in high predatory environments, the color patterns are strictly Y-linked, whereas in predatory low environments the patterns are also found on the X-chromosome (Haskins et al., 1961). This phenomenon remains to be explained (Houde, 1997).

Several components of their life history of the guppy, i.e., age and size at maturation, litter size and intervals, are genetically controlled. Two decades of investigations on guppies from Trinidad were conducted in situ as well as in laboratories, and provided evidence for rapid evolutionary adaptations of life history traits (Reznick et al., 1996a, 1996b, 1996c). Guppies from two different areas were investigated, i.e., from both the northern and the southern slopes the island of Trinidad. Both areas were divided into two regions, i.e., low predation upstream localities, and high predation downstream localities. Both the northern and the southern slopes exhibited the same results. In regions of high predation, guppies matured earlier and at a smaller size, had more and smaller offspring per litter, and had a higher reproductive allotment, than did guppies from the low predation regimes. From their experiments, Reznick and co-workers concluded that life histories, both phenotypically and genotypically, were influenced by predation pressure, i.e., resulted from mortality rates. Similar traits resulted from similar selection pressures produced by predation. Adaptations in regards to shoaling behavior, age and body size at maturation, number and size of offspring, reproductive allotments, female choice preferences, levels of aggression during foraging, and differences in pigmentation intensity were evident in these populations of guppies.

The evolutionary changes are very rapid in *P. reticulata*. A single guppy stock was separated in a large enclosure (Burger's Zoo, Holland), and was (unintentionally) divided into a population that was predated by snakebirds, *Anhinga melanogaster*, and one that was free of predation. It was shown that guppy populations already exhibit most of the traits observed by
Reznick et al. (1996a, b, c) after an eight year separation in a predatory and a non-predatory environment (Albers, 2000).

Character development in *P. reticulata* caused by food

Food availability is also an environmental agent producing morphological adaptations in guppies (Robinson and Wilson, 1995). A population of guppies was split in several subpopulations, and each subpopulation was subjected to one of the four different ways in which the food was presented:

i) Floating completely on the surface, like flake food  
ii) Floating with an end sticking into the water, like the pupae of mosquitoes  
iii) Floating in midwater  
iv) At the bottom with the end sticking up, like *Tubifex*

A control population was kept with a rotating scheme of these strategies.

The results were amazingly direct. Within a few generations, differentiation in body shapes was noticed and these morphological attributes were accompanied by behavioral traits. The different subpopulations began to behave like different species, with mating preferences for their 'own' subpopulation. This demonstrated that guppies can rapidly adapt to changes in their environment on population level, morphologically and in mating preferences.

Although it is possible that this mechanism is only present in guppies, it does provide evidence that morphological and behavioral differentiations are possible without actual genetical separation of populations.

Character development in *P. reticulata* caused by sexual selection

In an excellent monograph, Dr Anne Houde (1997) summarized 30 years of investigations on the correlation of sexual selection and developmental several traits in the guppy. Because the conspicuous colors of the males are an obviously predatory risk, its development must have other important advantages. One advantage is the attraction to females. In a series of experiments and examples, Houde (1997) described how this male-female interaction works. In combination with an elaborate mating behavior pattern, females are able to determine the fitness (the health, agility, etc.) of males by evaluating their color patterns and behavior. The
conflict between visibility for female and invisibility for predators is demonstrated by Endler (1980). Guppies raised in a non-predator environment had large spots when the gravel in their tanks was small and small spots when the gravel was large, to optimize their visibility. However, when a predator, viz., *Crenicichla alta* or *Rivulus hartii*, was present, the size of the spots on the guppy correlated positively with the size of the gravel in their tanks.

In the present thesis, a small contribution is made to the understanding of guppy color control (Chapter 11). In a new view on the genetics of guppy coloration, I hypothesize a new mechanism explaining the apparent contradiction between the fixed color patterns (cf. Winge, 1922, 1927) and the flexibility of their expression (cf. Houde, 1997).

7.2 Character displacement, or non-random character development caused by competing populations of closely related species.

Character displacement is defined as "the situation in which, when the areas of distribution of two species of animals overlap geographically, the differences between them are accentuated in the zone of sympatry and weakened or lost entirely in the parts of their ranges outside this zone" (Brown and Wilson, 1956). Futuyma (1979) considered character displacement as one of the "major undisputed features" associated with evolution. He stated that divergence of character development 'counteracts' three processes: interspecific competition, hybridization, and predation. This mechanism seems to have a large impact on character developments in *Poecilia* and I will, therefore, introduce this mechanism extensively.

The term character displacement is applicable to at least two different mechanisms causing character shifts (Loftus-Hills and Littlejohn, 1992):

i) Reinforcement caused by competition is a selective process that causes the accentuation of differences (ecological character displacement). In sympatry, two species are larger than in allopatry, for instance to be more able to compete for food. This latter mechanism can also cause convergent character displacement.

ii) Reproductive character displacement, which is applied to geographical variation in mating behavior, thus counteracts hybridization. For instance, specific markings on the body are more pronounced in sympatric populations than in allopatry.

Extensive research by Schluter and McPhail (1992, 1993) yielded several examples and criteria that indicated ecological character displacement that they believed was the driving force of adaptive radiation. Their evidence for 'species pairs' of sticklebacks caused by character displacement was threefold. They found:

1. Greater morphological and ecological differences between sympatric populations than between allopatric populations,
2. Nonrandom character distributions in coexisting sets of related species,
3. Exaggerated levels of divergence between new species formed in isolation from competing species.
This phenomenon occurs also in other, independent lineages of fish: whitefish, salmon and many others (Robinson and Wilson, 1994). Schluter and McPhail (1992) formulated six criteria to test character displacement:

1. Chance should be ruled out as an explanation for the pattern.
2. The phenetic differences should have a genetic basis.
3. Enhanced differences should be an intraspecific variation in characters; not simply a result of extinction of adapted (intermediary) species.
4. The examined phenotypic differences should reflect functional differences.
5. Sites of sympatry and allopatry should be comparable in ecological features, i.e., differences should only be attributable to interspecific interactions.
6. Independent evidence should be gained that similar forms actually compete for food.

Competition for food between newly formed species is a force with large potential impact.

Character displacement is not undisputed in every particular case. Fenchel (1975a, 1975b, in: Cherrill and James, 1987) examined three species of mud snails (genus: *Hydrobia*) and proposed character displacement for the observed size differences. Shell size is correlated to food particle size, so it was hypothesized that when competition occurs, related species will specialize in certain foods and will therefore be different in size. All examined species of snails appeared to have a biased size variation in sympatric populations, an indication for character displacement. However, this "textbook example" of character displacement has been adequately questioned by various authors (Cherrill and James, 1987; Saloniemi, 1993; Gorbushin, 1996). These authors showed that the character shifts in Fenchel's three species of snails could also be explained by habitat preference: the size differences, viz., the observed character shifts, could also be explained by habitat selection of the species. It appeared that the dominant species, which grows largest in a certain type of habitat, had its ecological optimum in sympatric populations. The size differences were attributed to environmental factors, not to interspecific competition and character displacement (Cherrill and James, 1987; Saloniemi, 1993).

Character displacement in fish

An extensive survey of fish literature was provided by Robinson and Wilson (1994), in which they gave examples for all six criteria from Schluter and McPhail (1992). One theoretical pitfall is the calibration of characters, i.e., identifying the original character states from which the observed character states deviated (Grant, 1972). If species have originated from known allopatric populations, these latter populations can generally serve as a control (cf. Rivas, 1982; Cherrill and James, 1987). If, however, species have evolved from sympatric populations, allopatric populations have no diagnostic value: these are possibly examples of character release, i.e., species show broader variation in characters in newly formed allopatric populations as opposed to (longer existing) sympatric populations. Moreover, it is practically impossible to attribute all differences in characters to the presence of another species. See for
instance a report of Schluter (1994, in: Grant, 1994) on induced sticklebacks differentiation, and its subsequent defense and attacks by various authors, i.e., Grant (1994), Murtaugh (1995), Bernardo et al. (1995), and Schluter (1995). Fundamental difficulties are threefold (Grant, 1972). First, how are original and derived populations identified? Second, how are pre-contact character states recognized? Finally, how does one find a zone of sympathy to identify character states in the absence of character displacement? I will return to the third question below, where an example is given by the Hubbs-Rivas mechanism (Section 7.2.3).

7.2.1 Character displacement in *Poecilia*

In poeciliids, feeding habits are reflected by dental types, e.g., large pointed teeth for an insectivorous feeding behavior versus small conical teeth for a more omnivorous feeding behavior. It is most probable that unicuspid inner teeth versus tricuspid inner teeth is also reflecting different feeding behaviors. At this point, one can in fact generalize and say that large pointed teeth are mostly associated with primitive genera, like *Priapella* and *Alfar* in the Poeciliinae, whereas the smaller dental types occur in more recent genera, like *Poecilia* and *Xiphophorus* (cf. Rosen and Bailey, 1963). In *Poecilia*, the smaller inner teeth are again subdivided into unicuspid, conical teeth and tricuspid inner teeth (providing more surface). This latter feature might be useful for grazers, i.e., for species that feed more on algae than on animal food.
In several cases, the shape of these inner teeth is the only reliable method of distinguishing between *P. sphenops* and *P. mexicana* (cf. Menzel and Darnell, 1973), especially in allopatric populations. In sympatric populations, more differences are noticed (Menzel and Darnell, 1973), indicating a form of character displacement for these two species.

An extensively documented case of character displacement was proposed by Rivas (1982) for *P. dominicensis* and *P. hispaniolana*. Rivas combined the interspecific differences with the geological history of Hispaniola, providing an excellent case of evolutionary biology based solely on alpha-taxonomy.

Interactions between *P. gillii* and *P. marcellinoi*

Discoveries of *P. gillii* "salvatoris" and *P. marcellinoi*

To test the applicability of the Hubbs-mechanism of character variation (subsequent to the investigations on *P. vandepolli* [cf. Poeser, 1992, Chapter 1 in this thesis]) I investigated a large quantity of mollies from El Salvador (cf. Poeser, 1995, Chapter 2 in this thesis). They were initially identified as a single species, viz., "*P. sphenops*" (cf. Boeseman, 1956). I started out to test whether meristic and morphological characters varied according to the predictions of Hubbs. Unlike in *P. vandepolli* and *P. gillii*, the variation of characters in the alleged *P. sphenops* contradicted Hubbs’ mechanism. The first inconsistency noted was that inland populations grew larger as opposed to coastal populations, although those environments are supposed to be less saline. The alternative conclusion would be that this variation was caused by temperature differences, i.e., inland populations occupy cooler environments. If so, the inconsistency in body sizes should also be correlated to variation in meristic features. That is, if inland conditions were cooler, a larger number of fin rays would be expected. However, no correlation was found, e.g., the mean number of dorsal fin rays remained about 9.5.

On closer inspection, some peculiarities began to emerge. In inland populations of this assembly of *P. sphenops*, the number of dorsal fin rays was ten when a dorsal fin spot was present, whereas it was nine when the dorsal fin lacked such pigment. Furthermore, in the populations without dorsal fin pigment a blotch on the caudal base was present. This indicated that in inland populations the number of dorsal fin rays was correlated with the position of a pigmented blotch. Subsequent examination revealed that the two forms differed in tooth shape: inland populations with 10 dorsal fin rays and a dorsal blotch had unicuspoid inner teeth and the inland populations with 9 dorsal fin rays and a caudal blotch had tricuspid inner teeth. The dental differences were also present in coastal populations, although the differentiation in pigmentation was less clear. Moreover, unicuspoid and tricuspid populations differed in standard length but not in dorsal fin ray number.

Character differentiation between *P. gillii* and *P. marcellinoi*.

Poecilia gillii and *P. marcellinoi* occur sympatrically in several habitats in El Salvador. To investigate geographical correlations between the species and their characters, the collection
sites from Boeseman (1956) were grouped into four regions (Fig. 5). These were two regions in the coastal areas and two regions more inland. Between these four regions, possible correlations between all examined characters were examined and 33 instances of intraspecific variations were recorded (Chapter 2). More than twice as much intraspecific difference occurred between an inland population and a coastal population, than intraspecific differences within inland populations or within downstream populations. All characters in *P. gillii* differ between coastal and inland areas, except depressed dorsal length and the basal length of the dorsal fin. Only standard length and the number of dorsal fins varied in *P. marcellinoi*.

![Figure 6](image)

**Figure 6.** Average dorsal fin ray numbers, showing character displacement in females of *P. gillii* "salvatoris" and *P. marcellinoi* in El Salvador. (1 = upstream riverine localities; 2 = fresh water lakes; 3 = downstream riverine localities, west of Rio Lempa; 4 = downstream riverine localities, east of Rio Lempa [After Poeser, 1995]).

In regions 1 and 2 most specimens of *P. gillii* have 10 dorsal fin rays, where *P. marcellinoi* has about 9 dorsal fin rays. In regions 3 and 4, both species have specimens randomly with 9 or 10 dorsal fin rays.

The unicuspid form was thereafter identified as *P. salvatoris* (cf. Miller, 1994, = *P. gillii* "salvatoris"), the tricuspid species was described as new, viz., *P. marcellinoi*.

Both species varied in the number of dorsal fin rays, but they both differentiate differently. *Poecilia gillii* has 10 dorsal fin rays in the inland regions, and 9-10 in the coastal regions, whereas *P. marcellinoi* has 9 dorsal fin rays in the inland regions, and (like *P. gillii*) 9-10 in the coastal regions (Fig. 6). A strong case of character convergence was present: the size difference between *P. gillii* and *P. marcellinoi* in the coastal zone was not longer significant
in inland populations (Fig. 7). To summarize, character displacement was obvious in variations in body size:

i) Both species showed intraspecific variation in size, coastal populations in both species were smaller compared to inland populations.

ii) Only in the coastal areas, *Poecilia gillii* is significantly larger than *P. marcellinoi*.

iii) In fresh water, there is no significant difference in size between the two species.

Character displacement was most obvious in the number of dorsal fin rays:

i) In coastal areas, the average number of dorsal fin rays is about 9.5 for both species.

ii) In fresh water habitats, *P. gillii* has 10 dorsal fin rays; *P. marcellinoi* has 9 dorsal fin rays.

![Figure 7. Averages of the Standard Length (SL), showing character displacement in females of *P. gilli* "salvatoris" and *P. marcellinoi* in El Salvador. (1 = upstream riverine localities; 2 = fresh water lakes; 3 = downstream riverine localities, west of Rio Lempa; 4 = downstream riverine localities, east of Rio Lempa [After Poeser, 1995]). Both species are larger in freshwater environments, minimizing size differences.](image)

The reduction in interspecific size difference confirms character shift reinforced by competition. Reproductive character differentiation counteracting hybridization reinforced character displacement in fin size and the position of the dark blotch. Differentiation by character displacement is enhanced in *P. gillii* and *P. marcellinoi* in El Salvador by their color patterns. *Poecilia gillii* has a black blotch in a red dorsal fin, whereas *P. marcellinoi* has a spotless yellow dorsal fin and a dark blotch at the caudal base.
Are there other mechanisms possible for the observed differences? Differences in size could result from a sampling bias. If, for example, the sampling of different populations occurred in different periods of the year, different age classes (and therefore size classes) could have been sampled, causing the observed variations. However, Boeseman (1956) reported that he found females containing young in different stages of development in all samples, so different year classes are ruled out.

Another feature causing genetically stable size differences in poeciliids is predation (Section 7.1). The presence of comparable (cichlid) predators throughout the country contradicts this possibility.

A closer look at the data presented for *P. gillii* revealed that intraspecific character differentiation follow the Hubbs-mechanism, i.e., the lower temperatures in inland populations produce bigger specimens with bigger meristic counts. *Poecilia marcellinoi* follows Hubbs' predictions for size only. Its meristic characters are precisely the opposite. Moreover, character developments in *P. gillii* have a reverse gradient compared to the variation found in *P. mexicana*, making it highly improbable that the Hubbs-mechanism, i.e., the temperature rather than salinity, is responsible for the variation in *P. gillii*. Downstream habitats were suspected to have marine influences. This suggestion is confirmed by syntopic faunal components like gobiids, indicating marine influences (Boeseman, 1956).

7.2.2 Re-examination of research data, making sense of character distribution in *Poecilia*

The recognition of character displacement as a factor related to character development in *Poecilia* made new interpretations of results from previous investigations possible.

**Interactions between *P. sphenops* and *P. catemaconis***

Examination of populations of *P. sphenops* near Sabia, Atlantic coast of Mexico, revealed that females have rows of spots on the sides of their bodies (pers. obs.), in contrast to the report of Schultz and Miller (1973). Most populations are spotless (confirming Schultz and Miller, 1973), but Sabia is near Lake Catemaco, which is inhabited by *P. catemaconis* and this tricuspid species also lacks spots on the sides of the body. The occurrence of spots in the females of these particular populations of *P. sphenops* might therefore be considered as a form of reproductive character displacement.

**Interactions between *P. sphenops*, *P. marcellinoi*, *P. pallida*, *P. nelsoni* and *P. marcellinoi***

Schultz and Miller (1971) did not distinguish between *P. butleri* and *P. nelsoni*, two unicuspid species with eight anal fin rays. They did, however, note that the populations north of Cabo Corrientes, Mexico, always have 18 scales around the caudal peduncle (= *P. butleri*), and that populations south of that point have 16 scales around the caudal peduncle (= *P. nelsoni*). These two species do not co-occur. The tricuspid species of the *P. sphenops* complex on the Pacific coast of Mexico are also distinguished by the number of scales around their tails, and they also do not co-occur: the species with 18 scale around the caudal peduncle
inhabits areas south of the Isthmus of Tehuantepec (= *P. sphenops*), the species with 16 scales around the tail (= *P. marcellinoi*) lives north of that border (Chapter 11). *Poecilia marcellinoi* does not occur in the Rio Balsas drainage, where the tricuspid *P. pallida*, with 18 scales around the caudal peduncle, occurs.

North of Cabo Corrientes, an unicuspid species, viz., *P. butleri*, has 18 scales around the caudal peduncle, whereas south of this point the unicuspid species, viz., *P. nelsoni*, co-occurs with either *P. marcellinoi* (tricuspid, also 16 scales around the caudal peduncle) or with *P. pallida* (18 scales around the caudal peduncle). The tricuspid species seem to not co-occur (Schultz and Miller, 1971). South of the Isthmus of Tehuantepec it is the tricuspid species that has 18 scales around the caudal peduncle (= *P. sphenops*, versus 16 in the unicuspid species, viz., *P. nelsoni*).

A third differentiating character, next to tooth shapes and caudal scale counts, is the pigmentation in the dorsal fin (Schultz and Miller, 1971). The populations in the Rio Papagayo basin, south of Cabo Corrientes, north of the Isthmus of Tehuantepec, can be distinguished by their dorsal fins: *P. nelsoni* has a ‘characteristic’ black blotch in an orange fin, *P. marcellinoi* has two or three parallel bands of black in a yellow fin. This is comparable to the situation in El Salvador, where *P. gillii* has a black blotch in a red fin, whereas *P. marcellinoi* has no black in a yellow fin.

Interactions between *P. marcellinoi*, *P. nelsoni*, *P. pallida*, and *P. chica*

Body colors are also possible differences among tricuspid species. In the Rio Balsas, an endemic form of tricuspid species occurs, viz., *P. pallida*. This species has 18 scales around the caudal peduncle. This is a pale species (pers. obs.), as opposed to *P. marcellinoi* and *P. nelsoni*. In the Rio Purificacion system, a small tricuspid species occurs, viz., *P. chica*, which is darker bodied than the co-occurring congeneric species.

7.2.3 The Hubbs-Rivas mechanism

Character displacement, formulated for *Poecilia* by Rivas (1982), predicts size differences and similar meristic characteristics in allopatric populations. Size convergence and meristic differentiation develop in sympatric populations.

The Hubbs mechanism is not sufficient to explain the peculiar distribution of characters that was encountered in El Salvador. While the inland (sympatric) populations show character displacement, i.e., actually behave like sympatric species, the coastal (sympatric) populations behave like allopatric populations. This was confirmed for *P. gillii* and *P. marcellinoi* populations from El Salvador in a comparison with a *P. gillii* population from Panama (pers. obs.). The coastal populations from El Salvador resembled the population from Panama more than the upstream populations in El Salvador. The observed character displacement is present in fresh water environments, in coastal environments no character displacement is present. That is, less differentiation was observed between coastal El Salvador populations and populations from Panama compared to coastal populations and inland populations in El
Salvador (see also Thomerson, 1966, in: Rivas, 1982, for a similar situation in Fundulus, i.e., lacking character displacement because of the "... unstable nature of their syntopic association").

According to the mechanism proposed by Hubbs (1926b), the fresh water populations are more receptive to the presence of a related species, hence, show Rivas' (1982) mechanism. I therefore propose to name this combined mechanism, i.e., character displacement under favorable conditions only, the Hubbs-Rivas mechanism. The Hubbs-Rivas mechanism solves one problem of Grant (1972: how to find a zone of sympathy to identify character states in the absence of character displacement). The species are sympatric throughout El Salvador but only show character displacement in inland populations.

A superficial test of the Hubbs-Rivas mechanism

The data from Poeser (1995) can reasonable stand the test of the six criteria formulated by Schluter and McPhail (1992; see above). First, the statistic analysis shows that the number of dorsal fin rays, as well as the variation in size, is significally correlated with the predicted values associated with character displacement.

Second, the genetic basis for character displacement is admittedly circumstantial. Genetic stability of established ecotypes, in respect to dorsal fin ray number and size has been demonstrated repeatedly in Poecilia (Schmidt, 1919; Hubbs, 1936; Menzel and Darnell, 1973; Reznick, 1996a, b, c), consistent with the predictions of Hubbs (1926b). Genetic drift is absent in the smaller rivers (cf. Feltkamp and Kristensen [1969] in their explanation of the genetic data in P. vandepolli), whereas upstream Rio Lempa populations are clearly different (cf. Menzel and Darnell [1973] for a similar situation in P. mexicana).

Third, several of the original lots contained samples of both species, i.e., the populations were syntopic. All differences encountered are thus ascribed to intraspecific variation rather than to extinction of intermediate species.

Fourth, the convergence in size is attributed to competitive (agonistic) behavior (Rivas, 1982). Differences in the position of a dark blotch are contributed to reproductive character displacement.

Fifth, the criterion of similarity of environments is only applicable in a "two-species-in-one-environment" situation.

Finally, all species of Poecilia are potential omnivores. Therefore, while food scarcity causes competition, it also provides opportunities for differentiation. A clue advocating the latter situation is the occurrence of a different inner teeth shape in the two species. The occurrence of tricuspid or unicuspid inner teeth is an adaptation to food, so this difference should be noted as a form of (established) character displacement.

2.3 Hybridization

Speciation through hybridization is a special mix of allopatric speciation and sympatric speciation. When populations have differentiated in allopatry, they can somehow become
united again. When the area of regained sympathy has special circumstances ("if a unique and discrete habitat exists" [Strickberger, 1995]), hybrids might be better adapted and replace the parental species. In some cases, the hybrid population can adapt several traits of the parental populations by subsequent inbreeding, i.e., introgressive hybridization.

The species mentioned in this section have ranges that coincide the ranges of their assumed parent species. All three hypothesized hybrid species occur on the Atlantic side of Mexico, indicating a former zone of hybridization between *P. mexicana* and their broad-finned relatives. All species in the present section are unicuspid.

Three hybrid Central American mollies

Regan (1913) combined the *P. sphenops* group with modally 9 fin rays and the sailfin mollies with 12 or more dorsal fin rays in the genus *Mollienesia*. He based this revision on the intermediate position of *P. formosa* and *P. petenensis* (= his *M. gracilis*), both having 10 or 11 dorsal fin rays. *Poecilia formosa* is a gynogenetic species, which was long suspected to be of hybrid origin (Hubbs, 1933) before genetic investigations (Schartl et al., 1995) confirmed this in great detail (Section 2.5.1).

Three species of mollies are supposed to have originated through hybridization, viz., *P. formosa* (= *P. mexicana limantouri* x *latipinna*), *P. petenensis* (= *P. mexicana?* x *kykesis*) and *P. latipunctata* (= *P. mexicana* x *latipinna*).

There are more studies that have shown occurrences of natural hybrids within the genus *Poecilia*. Hubbs (1936) reported a hybrid *P. mexicana altissima* x *velifera*, Miller and Schultz (1959, in: Darnell, 1962) a probable hybrid *P. mexicana* x *latipinna*, Schultz and Miller (1971) a hybrid *P. cf. sphenops* x *butleri* (= *marcellinoi* x *nelsoni*) and Menzel and Darnell (1973) reported *P. mexicana* x *sphenops* and *P. mexicana* x *latipunctata* hybrids.

In the light of the extensive evidence for the origin of *P. formosa*, it seems logical to consider hybridization as the origin of *P. petenensis*, the other meristic intermediate species (Regan, 1913). In lake Pétèn, an ancestral population of *P. mexicana* than has hybridized with an ancestral population of *P. kykesis*, the local broad-finned molly.

In addition to the hybrid origin of *P. petenensis*, a solution emerges for a peculiar result in the genetic relationship of *P. latipunctata* Meek, 1904. This species, which is clearly a member of the short-finned mollies (cf. Miller, 1983), is genetically similar to *P. latipinna*, a broad-finned molly (Schartl et al., 1995).

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From the Amazon river to the Amazon molly and back again: Chapter 7


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