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Vegetation and predators mediate color pattern frequencies in *Poecilia sphenops* Valenciennes

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Abstract. The purpose of the present study was to investigate the body color evolution in *Poecilia sphenops* under different environmental conditions (aquaria vs. Peţea Lake and Băile Felix spring) and to explain what is the destiny of a non-natural color pattern when the domesticated population is moved in the wild. We concluded color patterns evolve step by step from the ornamental full-black to the ancestral phenotype, in aquaria. Although the black phenotype is one generally limited to some domesticated strains, we found it highly and natural conserved in the wild. Our observations revealed a lot of vegetation in both Peţea Lake and Băile Felix spring, where floating leaves created a darkness underwater. The darker is the water in the lakes, the higher is the chance of a black fish to escape from predators. According to "natural selection theory", the black phenotypes of *Poecilia sphenops* could be conserved in the population, or even natural produced there. When predators preferentially prey on the less pigmented individuals, the black ones (both males and females) have an increased fitness. Consequently, according to "sexual selection theory" darker females with a preference for darker males will have a higher fitness than darker females which have no such a preference. Black males have an advantage to inseminate more females and produce more black progeny. In this way, the cumulative effect of natural and sexual selection maintain the mutant and fancy black phenotypes in the feral molly populations.

Key Words: black molly, color dynamics, natural selection, sexual selection, Peţea, Băile Felix, *Nymphaea*.

Tartalom: Jelenlevő dolgozatunkban bemutatjuk kutatásaink eredmenyait, amelyek célja az volt, hogy megfigyeljük a *Poecilia sphenops* (molly) testszinváltozását külömböző életekörülmények közt (akváriumokban, " Peţea" tóban, és a felixi fürdők forrásvizeiben), illetve a nemesités során elért szinváltozatok sorsa a szabadbahelyezes után. Következtetésainkben azt tudjuk állítani, hogy akváriumokban, a szineződési változás lassan vált át a nemesitett feketéből az eredeti szinezödesbe. Habár, a fekete szinezödes csak a nemesitett akváriumi populacioknal a leghansulyozottabb, megfigyeléseink során a vadonban is rámutathatunk erre a szinvaltozatra. A petai tóban, illetve a felixi fürdök forrásvizeiben az uszónövenyek nagyszámban figyelhetök meg. Ezek a növenyek megfékezik a vizbeli fénybehatolást, és igy a fekete hal, ragadozoi elol valo menekvése sokkal sikeresebb. A naturális-szelekcio elméletére alapozva a fekete *Poecilia sphenops* fenotipok populacioi, ezekben a természetes vizrendszerekben könnyen szaporíthatok és megörizhetök. Olyan esetekben amikor a halragadozok szivesebben fogyassztják a világosabb szineződésü egyéneket a fekete színű egyének, (úgy a hímek mint a nöstények) nagyobb állókepességgel rendelkeznek. Követetkezteteskeppen, a szexuális-szelekció elméletere alapozva, azt allíthatjuk, hogy a fekete szineződésü nöstények amelyek a sötet szinezödesü hím iránt nyilvanyitanak vozodast, nagyobb állókepesseggel fognak rendelkezni mint a sötét szinezödesü nöstények és amelyek nem rendelkeznek ezzel a himiránti vonzodással. A fekete hímek, az irantuk valo vonzodásnak köszönhetöen nagyobb arányban fognak hozzájárulni a fekete fenotipusu populació szaporodásához. A naturális, illetve a szexuális szelekció összesitett hatásai nzilvanvalóan hozzájárulnak a fekete fenotipusu mollyk fenntartásában a természetes életkörülmények közt.

Kulcsszavak: fekete molly, testszin evolucioja, természetes szelekciò, szexualis szelekciò, Peţea, felixi fürdök, *Nymphaea.*

Rezumat. Scopul prezentei lucrări a fost acela de a investiga evoluţia culorii corpului la peştele *Poecilia sphenops* în diferite condiţii de viaţă (acvarii vs. Lacul Peţea şi izvorul de la Băile Felix) şi de a înţelege soarta unor tipare coloristice non-naturale atunci când populaţia ameliorată este eliberată în natură. Am concluzionat că, în acvarii, tiparele coloristice evoluează treptat de la ornamentalul negru complet spre fenotipul ancestral, de tip sălbatic. Deşi fenotipul negru este unul, în general, limitat la varietăţile de acvariu, noi l-am constatat puternic şi spontan conservat în sălbăticie. Observaţiile noastre au dezvăluit o vegetaţie abundentă atât în lacul Peţea cât şi în izvorul de la Băile Felix, unde frunzele plutitoare creează un mediu întunecos sub apă. Cu cât luminozitatea este mai redusă, cu atât şansa unui peşte negru de a scăpa de prădători este mai mare. Conform teoriei „selecţiei naturale", fenotipurile negre ale speciei *Poecilia sphenops*

ar putea fi, aici, conservate în populaţie sau chiar produse natural. Atunci când prădătorii consumă preferenţial indivizii mai puţin pigmentaţi, indivizii negrii (atât masculi cât şi femeli) au un fitness crescut. În consecinţă, conform teoriei „selecţiei sexuale", femelele de culoare închisă cu preferinţă comportamentală pentru masculi închişi la culoare vor avea un fitness mai mare decât femelele de culoare închisă, dar fără o astfel de preferinţă. Masculii negrii prezintă un avantaj selectiv să insemineze mai multe femele şi să producă mai multă progenitură neagră. În acest fel, efectul cumulat al selecţiei naturale şi selecţiei sexuale menţin fenotipurile ornamentale negre în populaţiile sălbatice de peşti molly.
Cuvinte cheie: molly negru, dinamica coloritului, selecţie naturală, selecţie sexuală, Peţea, Băile Felix, *Nymphaea*.

Introduction. *Poecilia sphenops* (molly) is a tropical, benthopelagic, non-migratory, freshwater or brackish fish originary from Central America (Mexic to Colombia). The black variety (black molly) is a very popular aquarium fish and is marketed throughout the world. Molly is omnivorous, feeds on worms, crustaceans, insects, their larvae and green algae. In the aquarium it accepts also dried food, flakes and others. Water parameters: pH range - 7.5-8.2, dH range – 11.0-30.0, temperature - 18-28°C. Minimum population doubling time is less than 15 months. The male is smaller, with gonopodium; it has a maximum adult size of 6 cm TL, but sometimes larger in aquaria or ponds: 10 cm TL (especially in veiled stains). Males fight to establish a rank hierarchy. Females are bigger: 10 cm TL, but sometimes larger in aquaria or ponds – 15 cm TL (especially in veiled stains). *Poecilia sphenops* is an ovoviviparous fish, reproduction being possible anywhere and anytime. Females, generally give born to 5-20 young in a brood many times per year, but large females can produce even 40-50 young in a brood. In the last decades *Poecilia sphenops* and its ornamental hybrid varieties have spread in many tropical and thermal waters from all over the world (data compiled from: Woodhead & Armstrong (1985); Wischnath (1993), Hieronimus (1993); Bud 2002; I. V. Petrescu-Mag, unpubl. data). This poeciliid, as many others (Liley 1966; Piferrer & Lim 1997; Grether 2000; Bourne et al 2003; Hudon et al 2003; Karino et al 2005; Petrescu-Mag 2008), exhibits a sexual dimorphism due to the more pigmented bodies in males.

The goal of the present study was to investigate the body color evolution and dynamics in *Poecilia sphenops* under different environmental conditions. Another aim of the study was to understand and explain what is the destiny of a non-natural color pattern (the black body phenotype) when the domesticated population is moved in the wild.

Material and Method. Two feral and three domesticated populations of *Poecilia sphenops* were analyzed during several generations under different environmental conditions.

The two feral populations are located in Bihor County (north-western Romania, European Union): the former lives in Băile Felix Thermal spring (in a few small lakes in a park) and the latter in Peţea Lake (Natural Reservation; Băile 1 Mai). The last one is not older than 40-50 years (P. Bănărescu, unpubl. data), and we have very poor information about the temporal origin of these two molly populations. However, in both cases, the first feral mollies have escaped from aquarium or they were intentional introduced by aquarists in order to create a tropical and exotic appearance. Both populations are characterized by surprisingly large black body areas and many entirely black individuals (hypermelanism).

The three identical domesticated populations of black molly were bred at Bioflux Hatchery (SC Biofux SRL), USAMV-C Biobase, and Aquarium USAMV-C respectively. All the three populations were formed initially (beginning with 2006) by more than 99% entirely black individuals.

During 2006, 2007, and 2008 the three domesticated molly populations, and the two feral ones were observed from different points of view: ratio of different color patterns, color patterns dynamics in percentages, and ecological factors acting upon them.

Five color patterns were considered as formally basic phenotypic categories: (1) entirely black; (2) black body, grey eyes; (3) black with olive or yellow; (4) olive or yellow, spotted with black; (5) olive or yellow to whitish. The first two categories (1 and 2) can be considered black-molly, while the last three categories (3, 4 and 5) are more likely wild molly phenotypes. The above mentioned categories were ordered after the black pigment abundance (1>2>3>4>5).

Physical and chemical water parameters in aquaria were adjusted to be closely to those registered and/or reported (Berindei & Pop 1972) from the wild studied areas (pH=7.1±0.3; 28±4ºC; under a 14/10 hours light/dark cycle). Aquaria (100 to 400 l) were

plenty of submersed vegetation, well illuminated, where mollies were kept together with small numbers of swordtails (*Xiphophorus hellerii*, Heckel) and guppies (*Poecilia reticulata*, Peters). Net cages (20 cm x 15 cm x 15 cm) were used for fish keeping and reproduction. All the progeny produced by females were bred among parents beginning with the 10th day after birth. The old generations were step by step replaced by the young without any kind of artificial selection. Frequencies of different color patterns were estimated in percentages (similar to phytosociological relevee) from the total number of fish observed yearly (Figure 1). Detailed results sorted on phenotypic categories and their percentages were included in Table 1 and compared with results observed in the wild (results presented in the same table).

Results and Discussion. As we can see in the Table 1 and Figure 1, the percentage of black individuals seems to decrease significantly in aquaria beginning with 2006 and ending with 2008. The color pattern evolves step by step from the ornamental full-black to the ancestral wild phenotype (grey-olive color, yellow, or even whitish).

In the wild, the situation seems to be much different: although the black phenotype (2) is one generally limited to some domesticated strains, we found it highly and natural conserved in the feral populations from both Peţea Lake and Băile Felix spring (Table 1; Figure 1 and 2).

Table 1

Color pattern frequencies in three domesticated and two feral molly populations

Color pattern / *Population*	*Surface of water covered by leaves (%)*	*Entirely black (%)*	*Black body, grey eyes (%)*	*Black with olive or yellow (%)*	*Olive or yellow, spotted with black (%)*	*Olive or yellow to whitish (%)*
Bioflux Hatchery, 2006	0	100	0	0	0	0
Bioflux Hatchery, 2007	0	45	50	5	0	0
Bioflux Hatchery, 2008	0	20	30	20	29	1
USAMV-C Biobase, 2006	0	99	1	0	0	0
USAMV-C Biobase, 2007	0	40	50	9	1	0
USAMV-C Biobase, 2008	0	15	20	35	29	1
Aquarium USAMV, 2006	0	99	1	0	0	0
Aquarium USAMV, 2007	0	35	35	25	5	0
Aquarium USAMV, 2008	0	20	30	20	29	1
Peţea Lake, 2006	50	0	60	20	20	0
Peţea Lake, 2007	50	0	70	20	10	0
Peţea Lake, 2008	50	0	70	20	10	0
Băile Felix, 2006	50	1	74	20	5	0
Băile Felix, 2007	50	1	74	15	10	0
Băile Felix, 2008	50	0	75	20	5	0
Felix – the circular lake, 2006	80	0	90	5	5	0
Felix – the circular lake, 2007	80	0	90	5	5	0
Felix – the circular lake, 2008	80	1	89	5	5	0

Frequencies were estimated periodically in percentages (similar to phytosociological relevee).

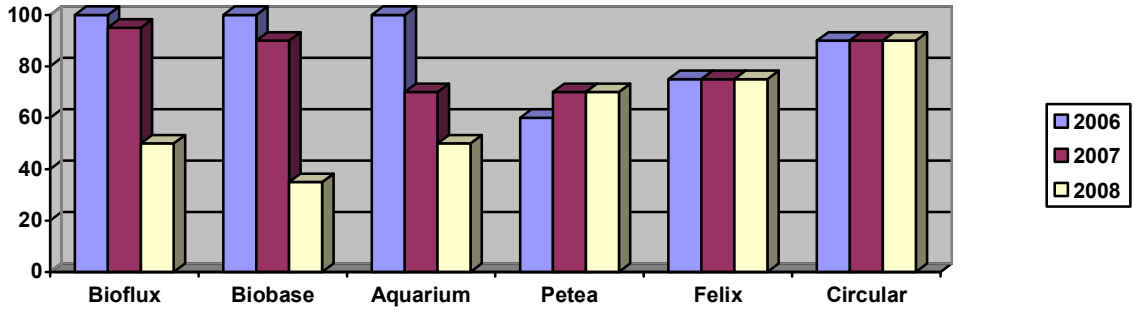

Figure 1: Evolution of frequency (%, on a vertical scale) of black phenotypes (1+2) from 2006 to 2008 in the wild (Petea, Felix) and captivity (Bioflux Hatchery, USAMV Biobase, Aquarium USAMV).

From the five phenotypes, the black one with grey eyes (2) appears to be most frequent in the wild (see Figure 2). The results included in Figure 2 were collected in 2008, after at least three years of natural selection in the wild (Peţea Lake and Băile Felix spring), and after three years of natural selection in captivity (Bioflux Hatchery, USAMV Biobase, Aquarium USAMV).

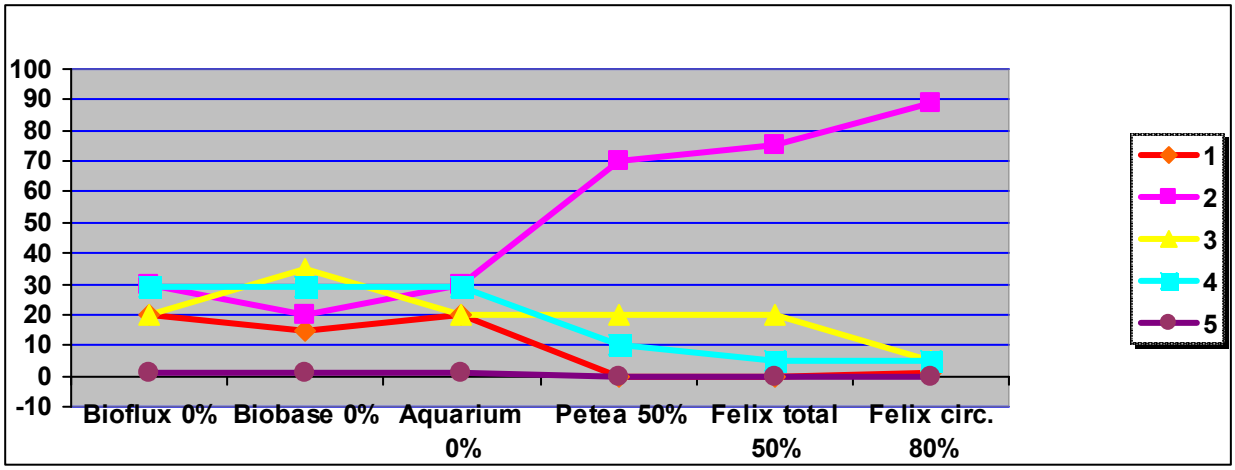

Figure 2: Frequencies (%, on the vertical scale) of the five different phenotypes (1-5) evaluated in 2008 in the wild (Petea, Felix) and captivity (Bioflux Hatchery, USAMV Biobase, Aquarium USAMV). Water surfaces covered by floating leaves are presented (%, on a horizontal scale) after the name of each location.

Generally, predators are natural "forces" selective acting over dynamics of the color traits and over poeciliid population behavior (Seghers 1974; Farr 1980; Seger 1985; Bischoff et al 1985; Nicoletto 1993; Endler & Houde 1995; Houde 1997; Brooks & Endler 2001; Kodrick-Brown & Nicoletto 2001). However, ornamental or fancy phenotypes are always or nearly always detrimental to any feral individual due to the sexual selection (Breden 1988; Basolo 1990, 1995; Bisazza 1993; Lindholm & Breden 2002), natural selection (Clark 1987; Endler 1980), and exposure to predators (Breden & Stoner 1987; Crawley et al 1991; Dugatkin 1992; Dugatkin & Godin 1992). The black individuals of *Poecilia sphenops* should decrease in percentage, due to a presumably female natural preference for grey-olive or yellow (wild type) males and due to their unusual body coloration that could make them an easier prey in the populations with predation risk (Winemiller et al 1990; Houde 1994). Corroborated with the female preference and predators, another important "force" that should lead to degeneration of recessive black phenotype in the molly is the dominance of wild alleles (Fisher 1930a,b).

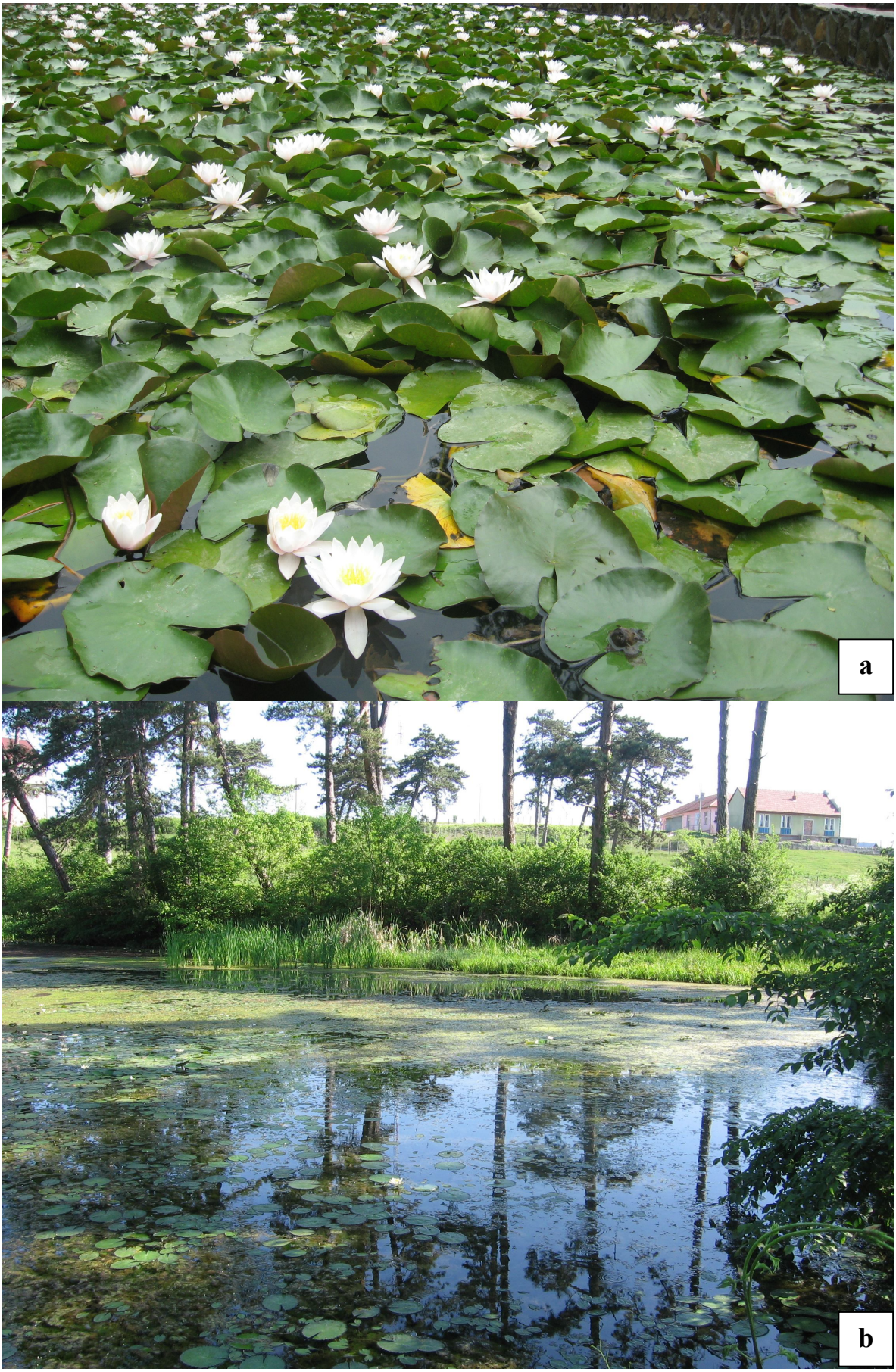

Plate 1. The water surface in the circular water body from Băile Felix (a) and Peţea (b).

In our feral populations, the percentage of black individuals was constant for three years: about 60-70% in the Peţea Lake, and about 75% at Băile Felix. What is the explanation for the intense and conserved skin melanism observed in the wild, while aquarium and hatchery results indicated a strong tendency of mollies to lose their black pigmentation from one generation to another? There is no other explanation for this situation than a higher fitness of the black individuals in some circumstances compared to wild-type (non-mutant) phenotypes.

Particularly, an isolated circular water body of the thermal spring from Băile Felix presented as much as 90% (or even more) black individuals, significantly higher compared to only 70% black individuals registered in the rest of Băile Felix water bodies. These findings have led to a pertinent explanation to the question we have raised above. Our observations revealed a lot of vegetation in both Peţea Lake (Plate 1, (b)) and Băile Felix spring. About 80% from the circular water body was covered by floating leaves (see Plate 1, (a)), and more than 50% from water surface of the other lakes was covered by leaves of *Nymphaea lotus* var. *thermalis*, Tuzs (Magnoliophyta, Nympheaceae; Tertiary relict (Olteanu-Cosma 1977)) and other close related floating plants. Furthermore, several exotic, tropical plants, such as: *Miriophyllum, Ambulia*, *Cabomba*, *Ceratopteris*, *Najas,* and a few others were present there, which additional to floating leaves created a darkness underwater. This ambiental condition can easily lead to conservation of hypermelanic phenotypes (see theories of Darwin 1859, 1871) despite of their domestic origin, only if there are important numbers of predators in the small thermal water lakes and springs. Mag et al (2005) reported on presence of the paradise fish *Macropodus opercularis* (Linnaeus), and brown bullhead *Ameiurus nebulosus* (Lesueur), in the Peţea Lake. The latter seems to be extinct in Peţea Lake, and the former was recently reported in small numbers from both Peţea Lake and Băile Felix spring (Iacob & Petrescu-Mag 2008). However, *Macropodus opercularis* is a small fish, generally unable to prey on *Poecilia sphenops* (excepting molly fries). Very probably, large adult *Xiphophorus hellerii* are most important fish predators there, which can be found in high numbers (Iacob & Petrescu-Mag 2008; I. V. Petrescu-Mag, unpubl. data).

All thermal waters from Bihor County have an additional and abundant predator: the Californian red-eared pond slider (*Trachemys scripta* var. *elegans,* Seidel), a voracious fish consumer. Description of the species (Bringsøe 2006): female has a maximum carapace length of 28 cm, males are smaller. They have a transverse yellow bar on each pleural scute, a plastral pattern consisting of a dark blotch or an ocellus on each scute, on a yellow ground colour and a wide red, or sometimes orange, stripe behind the eye. As the stripe continues further down the neck in a light color, it is reduced to a width of about one fourth. This stripe right behind the eye is considerably wider than the stripe which extends downwards behind the lower edge of the eye to the throat. The species has a narrow chin stripes (see Plate 2). This chelonian species is a non-native one for Romania. Another chelonian predator we found in small numbers in both Peţea and Băile Felix is *Emys orbicularis* (Linnaeus). This one is native to that geographical area (see Plate 3).

The darker is the water in the lakes, the higher is the chance of a black fish to escape (a case of natural selection; Darwin 1859). According to this theory, the black phenotypes of *Poecilia sphenops* could be conserved in a population, or even natural produced. Ironic, the nature reproduces in this way the man-made black molly, and copies the artificial selection. When predators preferentially prey on the less pigmented individuals, the black ones (both males and females) have an increased fitness. Consequently, darker females with a preference for darker males will have a higher fitness than darker females which have no such a preference (a case of sexual selection; Darwin 1871). In this manner, by natural and sexual selection combination, percentage of darker individuals can be maintained or increased in the wild.

However, the black eye phenotype (1) is almost absent, and probably due to the extreme melanism which in some doses could affect the health of the fish. Another explanation for the low frequencies of entirely black individuals is the recessive and polygenic nature of the mutant allele.

Plate 2. The most abundent chelonian predator from Băile Felix: *Trachemys scripta* var. *elegans*.

Conclusions. Percentage of black individuals of *Poecilia sphenops* seems to decrease significantly in aquaria beginning with 2006 and ending with 2008. The color pattern evolves step by step from the ornamental full-black to the ancestral wild phenotype. In the wild, the situation is different: although the black phenotype is one generally limited to some domesticated strains, we found it highly and natural conserved in the feral populations from both Peţea Lake and Băile Felix spring.

Generally (in the wild), predators are natural "forces" selective acting over dynamics of the color traits and over poeciliid population behavior. The black individuals of *Poecilia sphenops* should decrease in percentage, due to a presumably female natural preference for grey-olive or yellow (wild type) males and due to their unusual body coloration that could make them an easier prey in the populations with predation risk. Corroborated with the female preference and predation risk, another important "force" that should lead to degeneration of recessive black phenotypes in the molly is the dominance of wild alleles.

In the case of our feral populations, the percentage of black individuals was constant during 2006, 2007 and 2008: about 60-70% in the Peţea Lake, and about 75% at Băile Felix. There is no other explanation for this situation than a higher fitness of the black individuals under some circumstances compared to wild-type (non-mutant) phenotypes.

Plate 3. *Emys orbicularis* – a native chelonian, but not so abundant, in Peţea and Felix.

Particularly, an isolated circular water body of the thermal spring from Băile Felix presented as much as 90% black individuals, significantly higher compared to only 70% black individuals registered in the rest of Băile Felix water bodies. Our observations revealed a lot of vegetation in both Peţea Lake and Băile Felix spring. About 80% from the circular water body was covered by floating leaves, and more than 50% from water surface of the other lakes was covered by leaves of *Nymphaea lotus* var. *thermalis*, and other close related floating plants. Furthermore, several exotic, tropical plants, such as: *Miriophyllum, Ambulia*, *Cabomba*, *Ceratopteris*, *Najas,* and a few others were present there, which additional to floating leaves created a darkness underwater. This ambiental condition can easily lead to conservation of hypermelanic phenotypes despite of their domestic origin, only if there are important numbers of predators in the small thermal water lakes and springs. *Macropodus opercularis* was recently a predator reported in small numbers from both Peţea Lake and Băile Felix spring. However, *Macropodus opercularis* is a small fish (not so abundant), generally unable to prey on *Poecilia sphenops* (excepting molly fries). Perhaps, large adult *Xiphophorus hellerii* are most important fish predators there, which can be found in high numbers.

Thermal waters from Bihor County have all an additional and abundant predator: the Californian red-eared pond slider (*Trachemys scripta* var. *elegans*), a voracious fish consumer. The darker is the water in the lakes, the higher is the chance of a black fish to escape (a case of natural selection). According to this, the black phenotypes of *Poecilia sphenops* could be conserved in a population, or even natural produced. Ironic, the nature reproduces or conserves the man-made black molly and copies the artificial selection. When predators preferentially prey on the less pigmented individuals, the black ones (both males and females) have an increased fitness (the first chance to live and produce offspring). Consequently, darker females with a preference for darker males will have a higher fitness than darker females which have no such a preference (a case of sexual selection). Black males have the chance to inseminate more females and produce black progeny. In this way, the cumulative effect of natural and sexual selection maintain the black phenotypes in the population.

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