Genetically distinct colour morphs of European perch *Perca fluviatilis* in Lake Constance differ in susceptibility to macroparasites

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The unusual yellow-finned morph of European perch *Perca fluviatilis* found in Lake Constance suffers more severely from macroparasite infections, including the tapeworm *Triaenophorus nodulosus* and the gill worm *Ancyrocephalus percae*, than conspecifics elsewhere. Microsatellite analysis of yellow-finned *P. fluviatilis* and red-finned variant recently discovered in Lake Constance revealed significant genetic differentiation. Red-finned *P. fluviatilis* and fish with mixed fin colour, suggested backcrosses between red and yellow-finned colour morphs, exhibit better resilience to parasite infection, suggesting that the inability of the yellow-finned morph to reject macroparasites may have a genetic basis.

Key words: *Ancyrocephalus percae*; Cestoda; isthmus; Monogenea; *Triaenophorus nodulosus*; yellow fins.

At around 535 km^2, Lake Constance (47° 30′ N; 09° 30′ E) is one of the largest lakes in Europe (Petri, 2006). The large commercial fisheries on the lake target mainly whitefish, but catches of the European perch *Perca fluviatilis* (L. 1758) are also economically important (Eckmann & Rösch, 1998). *Perca fluviatilis* in Lake Constance are affected by various endo and ectoparasites (Balling & Pfeiffer, 1997*a*, *b*, *c, d*; Behrmann-Godel, 2013), including the extensively studied tapeworm *Triaenophorus nodulosus* (Dieterich & Eckmann, 2000). Plerocercoids of *T. nodulosus* utilize *P. fluviatilis* as second intermediate hosts, within which they infest the liver and are normally encapsulated after some weeks (Kuperman, 1973). Previous studies indicate that the established *P. fluviatilis* stock of Lake Constance is unusually susceptible to infection by *T. nodulosus*. Both prevalence and intensity of infections are higher than are usually seen in other circumpolar populations, and *T. nodulosus* in Lake Constance *P. fluviatilis* attain longer body length and diameter than in conspecific hosts elsewhere

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TABLE I. Differences in several aspects of *Triaenophorus nodulosus* and *Ancyrocephalus percae* infestation of adult yellow-finned *Perca fluviatilis* from Lake Constance compared with conspecifics from other lakes, found in the literature

	T. nodulosus		*A. percae*	
	Lake Constance[a]	Literature	Lake Constance[b]	Literature
Parasite length (mm)	46 ± 38 (mean ± s.d.) (maximum: 300)	10–100[b,c]	0·8–3·7	1·0–1·8[e]
Prevalence (%)	94	10–91[b,c,f–k]	85	1·1[g]
Mean intensity	4·2	1·5–4·3[g,j,k]	12–53	1·8[g]
Maximum intensity	52	3–28[b,f–h,j,k]	215	5[g]
Infestation site	Liver	Liver[c,d,f–k]	Isthmus[d,l]	Gills[e,g,m, n]
Rare sites	Other viscera, fillet	Other viscera[b,f,g]		
Damage	Severe	Moderate[b,h]	Severe	Mild[g]

[a]Brinker & Hamers (2007); [b]Kuperman (1973); [c]Hoffmann *et al.* (1986); [d]this study; [e]Ergens (1966); [f]Chubb (1964); [g]Andrews (1979); [h]Lucky & Navratil (1984); [i]Negele *et al.* (1990); [j]Morozinska-Gogol (2013); [k]Dezfuli *et al.* (2014); [l]Behrmann-Godel *et al.* (2014); [m]Bylund & Pugachev (1989); [n]Morozinska-Gogol (2008).

(Table I). Severely infected individuals exhibit markedly reduced growth, 16% down on uninfected conspecifics, and risk of mortality following spawning is significantly higher (Brinker & Hamers, 2007). Molzen (2005) observed a 20% reduction in fecundity of infected *P. fluviatilis* in Lake Constance, correlated with parasite-induced liver pathology.

The recent arrival in Lake Constance of an invasive gill worm of the genus *Ancyrocephalus* (Monogenea) prompted morphological and genetic studies that identified the species as *Ancyrocephalus percae* (Behrmann-Godel *et al.,* 2014). This parasite has a pan-European distribution (Andrews, 1979; Bylund & Pugachev, 1989; Morozinska-Gogol, 2008), and is not generally known for causing severe harm to its *P. fluviatilis* hosts (Andrews, 1979). Normally, *A. percae* infects only the gills of *P. fluviatilis* (Ergens, 1966) but in Lake Constance the adult parasites appear to actively migrate from the gills to the isthmus, where they cause deep, oval to round lesions (Behrmann-Godel *et al.*, 2014). In the most severe cases, the isthmus is perforated or even completely destroyed. This new target site and the resulting damage in *P. fluviatilis* hosts are, as far as is known, unique to Lake Constance (Table I). High intensity infections are normally quite unusual for *A. percae* (Andrews, 1979), but in afflicted Lake Constance *P. fluviatilis*, the number of parasites found infesting wounds on the isthmus can exceed 200 individuals (Behrmann-Godel *et al.*, 2014).

Historically, *P. fluviatilis* in Lake Constance have been characterized by yellow fin colour [Fig. 1(a)]. Recently, however, a red-finned morph has been observed. In laboratory studies, attempts to modify fin colour by supplementing dry food with carotenoids, resulted in only a slight increase in intensity of the yellow colouration, the fin colour could not be changed to red (R. Eckmann, unpubl. data). In addition to fin colour, the two morphs also exhibit differences in body shape and colour pattern of the body. Mapping the distribution of the new red-finned morph requires a lake-wide study, yet to be carried out, and their life cycle has not yet been examined. Since in 2013,
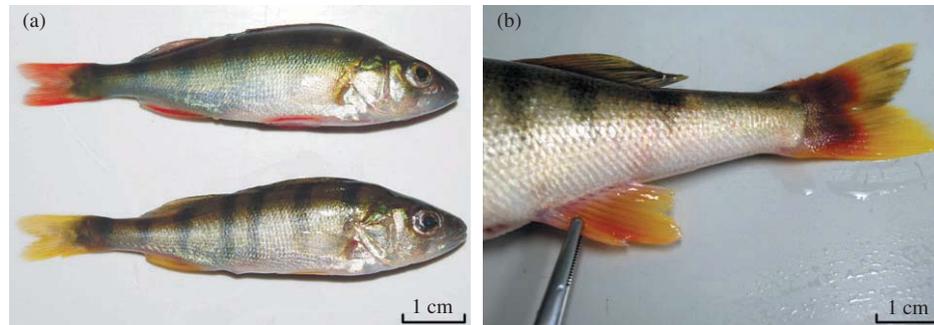
FIG. 1. Different fin colour morphs of *Perca fluviatilis* in Lake Constance. (a) Young-of-the year fish with red and yellow fin colour. (b) Adult yellow-finned fish with red patches in its fins (mixed colour morph).

however, adult fish have been recorded, with clearly defined red areas in their yellow fins, suggesting that cross-breeding between yellow- and red-finned morphs may be taking place [Fig. 1(b)]. These red patches were not related to infection or disease. In Langenargen marina (a marina located on the north shore of Lake Constance, Germany), juvenile *P. fluviatilis* with red fins can be caught on a regular basis. Early examinations of the *A. percae* infestation in 2012 indicated that yellow-finned *P. fluviatilis* are distinctly more affected by the gill parasite than their red-finned conspecifics. Taken together, these observations lead to the hypothesis that yellow-finned *P. fluviatilis* of Lake Constance lack certain genetic adaptations to cope with infections by endo and ecto-macroparasites, which consequently exhibit extraordinary virulence in these hosts.

For genetic characterization of the yellow, red and mixed fin colour *P. fluviatilis,* nine microsatellite loci in two multiplexes were analysed, using a Type-it kit (Qiagen; www.qiagen.com). The first batch contained the markers *PflaL2*, *Svi17*, *PflaL5*, *SviL7*, *Svi6* and *PflaL10* and the second batch contained the markers *Svi18*, *PflaL4* and *PflaL9* (Borer *et al.*, 1999; Wirth *et al.*, 1999; Leclerc *et al.*, 2000). The PCR cycle was 95° C for 5 min, then 27 rounds using a cycle at 95° C for 30 s, 56° C for 90 s and 72° C for 30 s, finishing with 60° C for 30 min. The two batches were fragment analysed on an ABI 3130 genetic analyser. Allele calling was carried out automatically using GeneMapper 4.0 (Applied Biosystems; www.appliedbiosystems.com) software, and controlled visually. Microsatellite data was checked for genotyping errors using Micro-Checker 2.2.3 (Van Oosterhout *et al.*, 2004). A total of 41 red-finned, 23 yellow-finned and 11 with mixed fin colour individuals of mixed age *P. fluviatilis* were successfully analysed, with no indication for large allele dropout, stuttering errors or null alleles. Possible deviations from the Hardy–Weinberg equilibrium (HWE) were analysed by comparing the number of expected and observed heterozygotes in the microsatellite data using Arlequin 3.5 software (Excoffier & Lischer, 2010). Deviations from HWE were found at five loci for red-finned *P. fluviatilis*, indicating further substructuring of these individuals, whereas none was found for the yellow-finned morphs. Heterozygosity and fixation indices (F_{ST}) were also calculated using Arlequin. The programme Structure 2.3.4 (Pritchard *et al.*, 2000) was used to visualize detailed population structure; Structure Harvester 0.6.8 (Earl & vonHoldt, 2012) was used to implement the Evanno method (Evanno *et al.*, 2005) to find the most probable number of clusters K.

The data revealed significant population differentiation (after Bonferroni correction) between yellow and red-finned morphs (pair-wise F_{ST} value of 0·08, $P < 0·001$; permutation test as implemented in Arlequin). Although only 11 individuals of the mixed-colour morph were caught and analysed, they differed significantly (after Bonferroni correction) from the red-finned morph (pair-wise F_{ST} value of 0·06, $P < 0·001$; permutation test as implemented in Arlequin). There was no apparent genetic differentiation in pair-wise comparisons of mixed colour and yellow-finned morphs (pair-wise F_{ST} value of 0·0015, $P > 0·05$; permutation test as implemented in Arlequin). Yellow-finned and red-finned *P. fluviatilis* appeared as two different groups in the Bayesian cluster analysis (Fig. 2) and a conclusion of $K = 2$ populations was strongly supported by the Evanno method. All but one yellow-finned individual exhibited high posterior probabilities for the yellow genotype. While most red-finned individuals yielded highest posterior probabilities for the red genotype, some also indicated high posterior probabilities for the yellow genotype or appeared intermediate. Nine of the individuals with mixed coloured fins exhibited high posterior probabilities for yellow and two for red genotype (Fig. 2). These results suggest that the red-finned group does not represent a pure genetic cluster. It is not known whether the red-finned morph has its evolutionary origin in Lake Constance, and thus it is impossible to define two pure reference populations for the purpose of exact hybrid status determination such as with NewHybrids analyses (Anderson & Thompson, 2002). An analysis without reference populations was inconclusive. It, therefore, remains unclear from the genetic information available whether or not mixed coloured *P. fluviatilis* might be backcross hybrids. The F_{ST} values and structural results, however, suggest that these fish are not F1 hybrids between red and yellow-finned *P. fluviatilis*, as this would have yielded intermediate F_{ST} values. In the light of all available information, the most probable explanation is that the mixed fin colour individuals caught in this study represent late backcrosses between red and yellow-finned morphs.

The situation existing in Lake Constance offered an excellent opportunity for comparing the parasite responses of local yellow-finned *P. fluviatilis* and the recently arrived red-finned morph. Both colour morphs are found in the same habitat, where they are exposed to both the established *T. nodulosus*, and the recently introduced *A. percae*. Between July and October, young-of-the-year (YOY) *P. fluviatilis* were sampled once a week, using sinking nets or electrofishing in the Langenargen (Germany) marina area. A total of 300 yellow-finned and 109 red-finned YOY were caught in the same habitat. Adult fish (2 years and older) were sampled once every 2 weeks, using a bottom net, with mesh sizes of 28 and 32 mm, at $10–30$ m depth near Langenargen. Adult red-finned *P. fluviatilis* ($n = 15$) and adult mixed colour morphs ($n = 13$) were much less common than adult yellow-finned fish ($n = 290$). The age of each individual adult *P. fluviatilis* was established by examination of the opercula bones (Le Cren, 1947). The livers of all fish were examined for signs of infection with *T. nodulosus*, according to Brinker & Hamers (2007), and variables of *A. percae* and *T. nodulosus* infestation were measured following Bush *et al.* (1997). Differences in the prevalence and intensity of infestation between colour morphs were analysed statistically using Fisher's exact tests and Wilcoxon tests, respectively, with significance levels set at 0·05 (Bosch, 1993). Welch tests were applied to test differences in variance between individual samples (Welch, 1938). The statistical analyses were conducted using JMP Pro 11.2.1 (SAS; www.jmp.com).
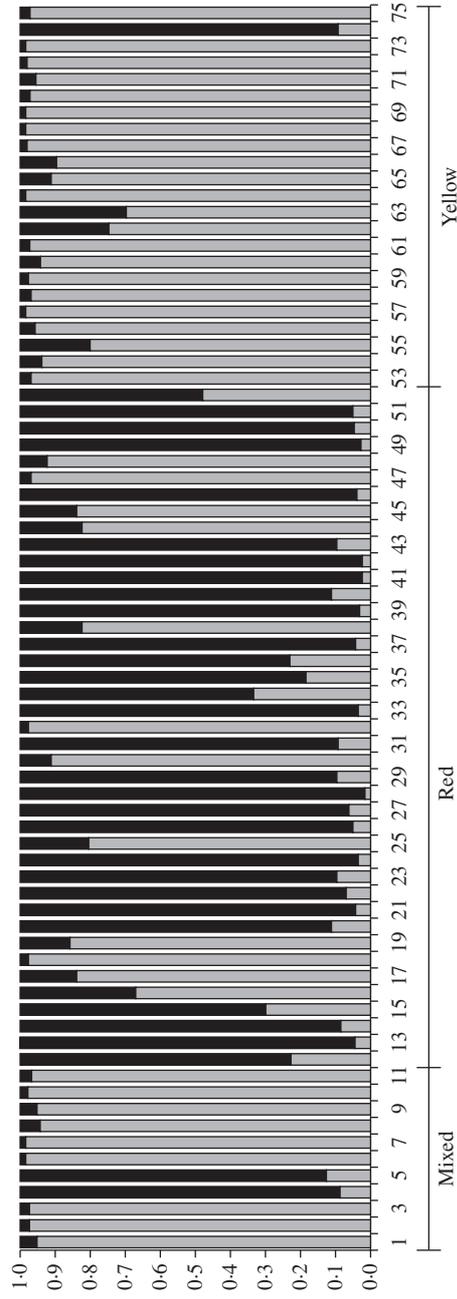
Fig. 2. Posterior probability (q) for ancestry of *Perca fluviatilis* from Lake Constance. Each individual is represented by a vertical bar, partitioned into segments that describe the membership proportion belonging to one of the two clusters defined by Structure analysis (■, red-finned genotype; □, yellow-finned genotype).
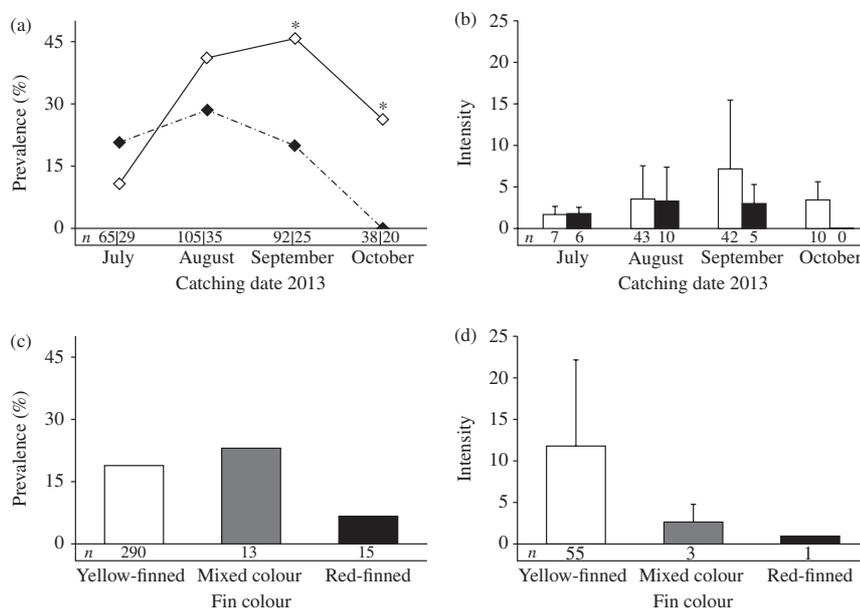
FIG. 3. (a, b) Infestation of young of the year yellow-finned (◇, □) and red-finned (◆, ■) morph *Perca fluviatilis* and (c, d) adult (2 years and older) yellow-finned (□), mixed-colour (▨) and red-finned (■) morph *P. fluviatilis* with *Ancyrocephalus percae* in Lake Constance, 2013. (a, c) Per cent prevalence. (b, d) Mean + S.D. intensity. Numbers indicate sample size [*n*, sample size; in (a) yellow-finned|red-finned]. *, Statistically significant differences between colour morphs (prevalence, Fisher's exact test; intensity, Wilcoxon test; $P < 0.05$).

The results show that YOY *P. fluviatilis* arrive in the littoral zone in early summer uninfected with *A. percae*, but that infection sets in rapidly thereafter, reaching peak prevalence of 45·7% in September [Fig. 3(a)]. Infection among red-finned YOY fish increased much more slowly, peaking at 28·6% in August and declining thereafter. By October, prevalence among red-finned YOY had returned to zero. In contrast, by October, 26·3% of the YOY yellow-finned *P. fluviatilis* were still infected, a value comparable to the peak prevalence seen in the red-finned morph. These differences in prevalence between the two colour morphs were statistically significant in September and October (Fisher's exact test, d.f. = 1, $P < 0.05$).

The intensity of *A. percae* infection in YOY yellow-finned *P. fluviatilis* also increased in mid-summer, but remained stable in red-finned fish [Fig. 3(b)]. The maximum intensity for yellow-finned hosts was 45 *A. percae* individuals per fish, whereas for red-finned hosts it was only 14 parasites. Unfortunately, however, these differences could not be confirmed statistically (Wilcoxon test, d.f. = 1, $P > 0.05$). The test power was low as variability in the number of parasites found per individual was high. In September, when the greatest differences in infection intensities between the fin colour morphs were observed, there was a significant difference in variance (Welch test, $F_{1,20} = 6.33$, $P < 0.05$), indicating clear differences in the degree and type of infection between yellow and red-finned *P. fluviatilis* in Lake Constance.

As all YOY fish shared the same shallow-water habitat with each other and with older conspecifics (Eckmann & Rösch, 1998), and *A. percae* actively seize their hosts

(Behrmann-Godel *et al.*, 2014), both colour morphs face the same risk of infection. The defence mechanisms in affected tissues of host fish play an important role in resisting infection, particularly with respect to gyrodactylid parasites (Buchmann & Bresciani, 1997). The immune responses of YOY fish, however, may be incompletely developed (Uribe *et al.*, 2011), leading to a relatively high prevalence of infection. The observed reduction in infections of YOY red-finned *P. fluviatilis* in late summer is evidence of successful rejection of *A. percae*, leading to zero infection in October. In contrast, yellow-finned fish appear incapable of raising an appropriate defence against the parasite. The exceptionally high infection intensities observed in some yellow-finned hosts from Lake Constance corroborate the hypothesis outlined above.

Supporting results were found in adult *P. fluviatilis* (2 years and older). Due to the small proportions of red-finned and mixed-colour morphs in catches, a seasonal comparison of infestation variables was not possible. For 15 adult red-finned fish examined, however, only one individual was infected by *A. percae* [Fig. 3(c)], and in this fish only a single parasite was found. It can be concluded that it resembles an incidental infestation. Some differences in infection intensity were recorded between yellow-finned and mixed colour fish [Fig. 3(d)], but the results were not statistically significant (Wilcoxon test, d.f. $= 1$, $P > 0·05$). Of special interest is the mixed colour morph of *P. fluviatilis*. If the individuals analysed in this study are backcrosses between yellow and red-finned morphs, as indicated by the genetic analysis, the data suggests that some part of the *A. percae* resistance seen in red-finned fish is transmitted to backcross offspring [Fig. 3(c), (d)].

The hypothesis that the two colour morphs of *P. fluviatilis* in Lake Constance differ in their susceptibility to parasite infestation finds further support in the prevalence data for *T. nodulosus* infection (Fig. 4). Red-finned YOY fish were significantly less affected by *T. nodulosus* than young yellow-finned fish. (Fisher's exact test, d.f. $= 1$, $P < 0·05$). The pattern is repeated in adult fish, where nearly all yellow-finned individuals were infested by plerocercoids, while fish with mixed fin colour were significantly less affected, and red-finned individuals exhibited the lowest prevalence (Fisher's exact test, d.f. $= 1$, $P < 0·001$). Previous work has shown that yellow-finned *P. fluviatilis* from Lake Constance suffer higher prevalences of *T. nodulosus* infection and grows much larger in the liver of yellow-finned hosts. Plerocercoids do not normally grow larger than 10–100 mm in the liver of hosts (Kuperman, 1973; Hoffmann *et al.*, 1986), but those in Lake Constance are known to achieve lengths up to 300 mm (Brinker & Hamers, 2007). At around 95%, the prevalence of infection in yellow-finned Lake Constance *P. fluviatilis* is also one of the highest recorded in the literature (Brinker & Hamers, 2007). The reason for this exaggerated virulence is unclear. Extensive chemotaxis assays (Falk *et al.*, 1980) reveal a significant positive chemotactic reaction of *P. fluviatilis* leucocytes in response to both *T. nodulosus* and *A. percae*, but no differences were observed between the different *P. fluviatilis* colour morphs. Intensive diet studies with the red-finned morph should now be carried out, examining possible preferences for certain copepod species. As first intermediate hosts for *T. nodulosus*, these prey items may account for differences in prevalence and intensity of infection in different *P. fluviatilis* colour morphs. Due to the ongoing reoligotrophication of Lake Constance, copepods dominate the zooplankton community (IGKB, 2011). Especially abundant are *Cyclops vicinus*, *Cyclops abyssorum*, *Eudiaptomus gracialis* and *Mesocyclops leuckarti* (Stich *et al.*, 2005), which all could serve as first intermediate hosts (Kuperman, 1973). The declining zooplankton abundance of oligotrophic
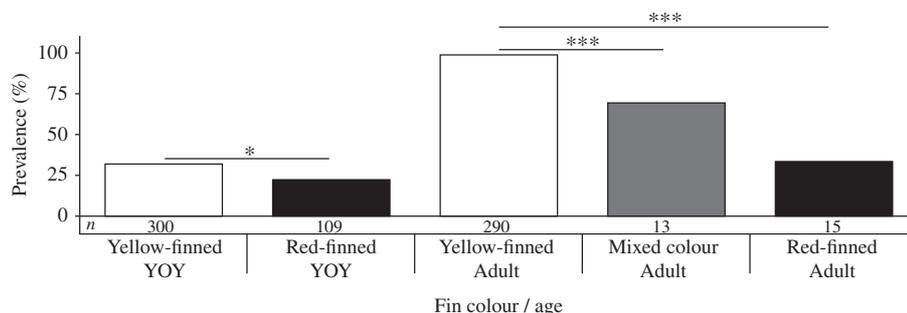
FIG. 4. Infestation of young of the year (YOY) and adult *Perca fluviatilis* [yellow-finned (□), mixed colour (▣) and red-finned (■) morph] with *Triaenophorus nodulosus* in Lake Constance, 2013. Prevalence is given as a percentage value. *n*, sample size. *, Statistically significant differences between colour morphs (Fisher's exact test, *$P < 0.05$; ***$P < 0.001$).

lakes increases the percentage of copepods infected with *T. nodulosus* (Lucky & Navratil, 1984) and thereby also increases the chances of any *P. fluviatilis* colour morph ingesting infected prey. Because all morphs share the same habitat, major differences in the copepod composition of their diet are thought to be unlikely.

In conclusion, these findings show clear differences between the yellow-finned and red-finned morphs of Lake Constance *P. fluviatilis*, with distinctions not only in fin colour, but also in their ability to handle macroparasite infections. The yellow-finned morph in Lake Constance appears unable to muster an adequate defence to either *A. percae* or *T. nodulosus*, resulting in a higher prevalence and intensity of infestation and targeting of the isthmus as a new infection site by *A. percae* (Table I). Both macroparasites are able to cause dramatic pathologic damage to yellow-finned *P. fluviatilis*, with severe ecological and economical effects on their stocks in Lake Constance. The mixed colour *P. fluviatilis* morph, with red patches in its yellow fins, appears intermediate between yellow- and red-finned individuals in terms of susceptibility to infection by both macroparasites, and may represent backcrosses between the two colour morphs, exhibiting traits from both parents. More genetic studies are needed to properly understand the relationship between the different colour morphs. These results raise fascinating questions, not least: (1) Why does the yellow-finned genotype remain so successful in Lake Constance, a waterbody surrounded and connected to systems with only red-finned *P. fluviatilis*? (2) How do the genotypes differ immunologically? and (3) What are the implications of *A. percae* selecting a new target tissue?

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