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How could fully scaled carps appear in natural waters in Madagascar?

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Abstract :

The capacity of organisms to rapidly evolve in response to environmental changes is a key feature of evolution, and studying mutation compensation is a way to evaluate whether alternative routes of evolution are possible or not. Common carps (Cyprinus carpio) carrying a homozygous loss-of-function mutation for the scale cover gene fgfr1a1, causing the 'mirror' reduced scale cover, were introduced in Madagascar a century ago. Here we show that carps in Malagasy natural waters are now predominantly covered with scales, though they still all carry the homozygous mutation. We also reveal that the number of scales in mutated carps is under strong polygenic genetic control, with a heritability of 0.49. As a whole, our results suggest that carps submitted to natural selection could evolve a wild-type-like scale cover in less than 40 generations from standing polygenic genetic variation, confirming similar findings mainly retrieved from model organisms.

Keywords : evolution, mutation compensation, heritability

Introduction

Reversibility of evolution is a long studied and questioned aspect of evolutionary biology[1]. Especially in small populations, slightly deleterious mutations may accumulate and become fixed by genetic drift[2]. To which extent and by which mechanisms the phenotypic effects of these mutations could be counteracted by natural selection is important in conservation biology, where individuals from small populations are re-introduced in nature, but also has more general implications on the mechanisms of adaptation. Although in some cases reversion of mutations has been observed[3], the general picture is more that mutations themselves are irreversible. However, reverse adaptation by compensation of deleterious mutations has been demonstrated experimentally, essentially in micro-organisms[4–7] and model invertebrate species[8,9]. The mechanisms invoked are diverse, but the appearance and/or selection of intermediate fitness compensatory mutations, not necessarily acting on the same biological pathways, seems more likely than reversion[5–7]. Many mutations remain cryptic (i.e. with little or no effect) in the absence of the deleterious mutation, and those cryptic mutations generate usable standing genetic variation that can be co-opted by natural selection, once revealed by the deleterious mutation[10]. Studies on the evolutionary basis of adaptation typically make use of organisms with rapid generation times and small physical size – in many cases microbes in a controlled environment[4,5,8,11]. Still, studies of natural populations remain particularly attractive as they can show evolution in action on macroscopic, easily scored traits, in complex organisms such as vertebrates[12–14], providing an ecological point of comparison for the artificial setups used in laboratory studies[15].

The common carp, *Cyprinus carpio* L., is a Cyprinid fish species originating from the Eurasian continent, which has a long history of domestication[16], and has also been introduced in many areas throughout the world[17]. While wild-type common carp are typically exhibiting a full scale cover, mutants in two independent bi-allelic Mendelian systems (S/s and N/n) have been selected during the domestication process, leading to four different scale patterns, the wild-type scaled phenotypes (genotypes SSnn or Ssnn), and reduced scale cover phenotypes identified as scattered or mirror (genotype ssnn), linear (genotypes SSNn or SsNn) and nude (genotype ssNn), all NN homozygotes being lethal[18]. The s allele of the S/s system has recently been shown to be a loss of function mutation in a kinase domain of *fgfr1a1*, which could be either a 310 bp deletion or a missense point mutation which encodes Lys-664 (AAA) instead of Glu-664 (GAA) in the *fgfr1a1* gene[19]. The N/n system has not been identified to date[20], although it shows some similarities with

mutations in ectodysplasin *eda* and its receptor *edar*, found in the zebrafish *Danio rerio*[21] and in the stickleback *Gaterosteus aculeatus*[22–24]. The N/n system may also be more complex than initially thought, and a gradation of phenotypes caused by dose-dependent signaling rather than a simple Mendelian bi-allelic system has been postulated[20].

Common carp of French origin was introduced to Madagascar in 1912 for fish farming purposes, and only the mirror phenotype was introduced, as the most valued for carp farming[25]. Between 1920 and 1950, carps quickly spread to most rivers and lakes, especially in the highlands and in the tropical lowlands of the Western coast of Madagascar[25,26]. At the end of the 1950s, field records showed many carps had "degenerated" to a scaled phenotype[25], and a new introduction of mirror carps from France took place in 1959 to "refresh" farmed stocks[27,28]. It is only in 1979 that carps with the wild-type scaled phenotype (strain Szarvas P33 – genotype SSnn) were introduced in Madagascar, together with other mirror carp (strain Szarvas 215 – genotype ssnn), from Hungary[29](Janos Bakos, pers. comm.).

In this study, we investigated the scale cover phenotype and the S/s genotype at *fgfr1a1* of common carps collected fish farms and in nature, in different areas of Madagascar. We also performed a controlled breeding experiment in common garden to investigate the segregation of scale cover phenotypes in the progeny of selected broodstock fish and estimate scale cover heritability.

Methods

Fish sampling

A total of 686 carps were sampled from 8 regions in Madagascar (406 from farms and 280 from the wild – see Supplementary Figure S1). For each fish sampled, origin (farmed/wild) and location were recorded, a digital picture was taken, and a fin sample was collected in 90% ethanol for further DNA extraction and genotyping. Phenotyping of scale cover was assessed on each picture first using a binary score: Scaled when the whole body was covered with scales, irrespective of their number, and Incomplete (mirror) when only part of the body was covered with scales. We will see later on that scaled carps may in reality encompass different phenotypes, the real wild-type scaly carp with >300 scales on one side, when fish carry at least one functional S allele, and a "fully-scaled mirror" type with < 300 scales when fish are s/s homozygotes.

Fgfr1a1 genotyping

Both regions from the kinase domain of *fgfr1a1* containing the two mutations – the EK substitution and the 310-bp deletion – previously reported as associated with a mirror phenotype in carp were investigated using the primer sets designed by Rohner *et al.*[19]. DNA was extracted with Wizard® Genomic DNA purification kit (Promega). PCR reactions were done for each sample in 4 wells with 2 µL of DNA solution (20 ng/µl), 3.63 µL of water, 2 µL of 5X GoTaq Flexi Buffer (Promega), 0.6 µL of MgCl2 (25 mM), 0.7 µL of dNTP (1 mM), 0.5 µL of each primer (10 µM) and 0.07 µL of GoTaq polymerase (5 U/µL, Promega). Thermocycling consisted of 5 min at 96°C, then 5 initial cycles of 30 s at 96°C (denaturation), 30 s at 58°C (annealing) and 1 min at 72°C (extension), followed by 25 cycles of 30 s at 96°C, 30 s at 58°C and 30 s at 72°C, and a final period of 5 min at 72°C. PCR products from 236 Malagasy carp samples were sent to Eurofins MWG (Germany) in order to perform a sequencing assay of the genomic region including the EK mutation at the first base of codon 664 in *fgfr1a1*. A subset of 45 samples was also explored for the presence of the 310-bp deletion that shortens intron 10 and exon 11 in some mirror carps. DNAs were visualized under UV light after migration at 130 V for 45 minutes in a 2% agarose ethidium bromide-stained gel. The whole amplicons had the expected size of around 550 bp, indicating the absence of deletion. In addition, 3 samples from 1 mirror and 2 fully-scaled mirror feral carps were submitted to sequencing and confirmed the absence of any polymorphism for the deletion allele. All sequences were analyzed using NovoSNP software[30].

Genetic structure of Malagasy carp populations

The 236 carps sequenced for *fgfr1a1*, as well a sample of 72 carps from Hungary, which were a contemporary sample of the populations introduced in 1979, were genotyped for 11 microsatellite markers HLJE265, HLJ2241, HLJ2346, HLJ2382, HLJ2465, HLJ2544, HLJ334, HLJ526, HLJ534[31], J58[32], MFW16[33] by Labogena (Jouy-en-Josas, France). To account for the validity of the set of microsatellite markers, F_{IS}[34] departure from Hardy-Weinberg equilibrium was tested through allele randomizations (10,000 permutations per test) using Fstat[35] within the five population samples with N≥30. Two of them (HLJ2346 and HLJ534) significantly departed from the Hardy-Weinberg equilibrium in at least one population after Bonferroni correction for multiple testing[36] (table S1), and were excluded from further analyses. Microsatellite genotypes were used for a clustering analysis with Structure[37], with an admixture model (default setting), correlated allele frequencies (default setting), 20.000 burn-in repetitions and 20.000 repetitions after burn-in. The most likely number of clusters K was assessed with the deltaK method[38], testing values of K ranging from 1 to 5 with 20 replicate simulations for each level of K. The allelic richness was computed with Fstat[35], and the number of private alleles was estimated in the farmed and feral samples from Madagascar and in the Hungarian farmed sample. All allelic data including those of the Hungarian carps were used to produce an unrooted tree, using an Unweighted Neighbor Joining (NJ) clustering method for dissimilarity matrix calculated by simple matching method [39] with 1000 bootstrap iterations implemented in DARwin6[40]. Genotype data are available in Supplementary Data 2.

Controlled breeding experiments

We also performed a controlled breeding experiment using carp broodstock collected in farms in the Vakinankaratra region (Tsiribihina drainage basin). Four males, two of which were fully-scaled mirror (FSM1, FSM2) and two of which were standard mirror (M1, M2) were mated to four mirror females in a full-factorial mating design, and 10 heterozygous S/s scaled males were mated to the same 4 mirror (s/s) females. Female ovulation was induced with Ovopel (D-Ala6, Pro9-Net-mGnRH, Unic-trade, Hungary) homogenized using 1 pellet/ml in 0.9% NaCl solution[41], using a first injection of 0.1 ml solution per kg of fish, and a second injection of 0.9 ml solution per kg of fish 12 hours later. Before any manipulation, the fish were anaesthetized with 2-phenoxyethanol (0.3 ml/l). Spawning occurred 12 hours after the second injection, and the spawns of the 4 females were stripped by gentle abdominal pressure

and mixed in equal volumes to produce a pool of eggs. The sperm of the 4 males had been collected 12 hours in advance by stripping, and was stored at 4°C in 5 ml syringes (max 1 ml sperm/syringe). Fifty grams of eggs from the pool were split in four equal parts of 12.5g, each being gently mixed with 0.1 ml sperm from one male, and activated with 15 ml of activation solution (3g/l urea, 4g/l NaCl). The operation was repeated with 50 g of eggs from the pool which were split into 10 equal aliquots of 5 ml, each fertilized with one heterozygous S/s male. One minute after activation, fertilization batches were mixed by sire type (mirror and fully-scaled mirror on one side, heterozygous scaled on the other side) and manually agitated with a semi-skimmed milk: water solution (1:4) for 30mn to avoid egg sticking, after which they were rinsed with hatchery water and each egg group incubated in a McDonald jar at an average temperature of 24°C. Hatching occurred at 47 hours post-fertilization, and larvae were transferred to a resorption tank with flow-through water. Two ponds were stocked with larvae, pond 1 (25 m²) with 800 larvae from the 4x4 cross and pond 2 (100 m²) with 1700 larvae, 425 from the 4x4 cross and 1275 from the 10x4 cross. At 109 days post-fertilisation, the ponds were drained, 363 fish were collected in pond 1 and 881 in pond 2. They were firs anaesthetized with 2-phenoxyethanol (0.3 ml/l), then a sub-sample was collected in each pond for further characterization: 74 fish in pond 1 and 486 in pond 2 were individually photographed (Canon Powershot S50), and a piece of fin was collected in 90% ethanol for further DNA extraction and parentage reconstruction.

Offspring and parents were genotyped for 14 microsatellite loci CCE46[42], HLJE265, HLJ2241, HLJ2346, HLJ2382, HLJ2465, HLJ2544, HLJ526, HLJ534, HLJ334[31], J58[32], KOI 57-58[43], MFW16, MFW40[33] by Labogena (Jouy-en-Josas, France). Parentage was assessed by exclusion with VITASSIGN[44], allowing for up to two allelic mismatches. 513 offspring out of 560 (91.6%) were assigned to a unique parental pair.

Numbers of scaled and mirror fish were counted in the offspring of the 10 heterozygous S/s males, and departure from 1:1 tested with a χ^2 test (data available in Supplementary Data 4). Six rice fields and four ponds were further stocked each with 50 scaled fish and 50 mirror fish at 110 days post-fertilisation, and survivors were counted and classified for scale cover at 11 months post-fertilisation. Departure from 1:1 was tested with a χ^2 test.

Acquisition and interpretation of scale numbers

Scales were counted on one flank on the digital pictures of 208 adult fish (feral and farmed) out of 236 that were genotyped and of 196 of the 197 offspring from the 4x4 controlled

breeding experiment that were unambiguously assigned to their parents. Individuals for which scales were not counted were removed due to low picture quality. This was done with ImageJ[45] using the Cell Counter plugin (http://rsbweb.nih.gov/ij/plugins/cell-counter.html). In the controlled breeding experiment, effects of sire, dam and their interaction were tested with the following mixed model in SAS:

$Y_{ijkl}=\mu+P_i+s_j+d_k+sd_{jk}+e_{ijkl}$

With Y_{ijkl} the number of scales in offspring l, μ the overall mean, P_i the fixed effect of pond i, s_j the random effect of sire j, d_k the random effect of dam k, sd_{jk} the random interaction term between sire j and dam k and e_{ijkl} the random residual. The same data were also used to estimate the heritability of scale cover using an animal model, with pond as a fixed effect, using VCE6[46]. Family and scale number data available in Supplementary Data 4.

Rates of evolution in KDarwins and haldanes were computed as proposed by Kinnison and Hendry[47]:

$$kd = \frac{\ln(x_2) - \ln(x_1)}{t}$$

Where *kd* is the rate of evolution in kilodarwins, $\ln(x_2)$ is the natural logarithm of the average number of scales in fully-scaled mirror carps, $\ln(x_1)$ is the natural logarithm of the average number of scales in mirror carps (taken as a surrogate for the initial number of scales in the mirror carps introduced inn1912), and t is the time interval in thousand years (here 0.1, or 100 years between 1912 and 2012)

$$h = \frac{{}^{x_2}/_{s_p} - {}^{x_1}/_{s_p}}{g}$$

Where h is the rate of evolution in haldanes x1 and x2 are as before, sp is the pooled standard deviation of scale number across mirror and fully-scales mirror groups, and g is the number of generations (here 40, taking a mean generation interval of 2.5 years).

Results

Occurrence of different scale patterns in feral and cultured carp populations

Of the 686 carps sampled from 4 river basins in Madagascar (406 from farms and 280 from the wild – see figure S1), 439 (64.0%) had incomplete scale cover and 247 (36.0%) were fully covered with scales. In farms, full scale cover was relatively uncommon (16.0%), while it clearly dominated (65.0%) in the wild. The situation in the wild varied greatly among sampling regions, with 93.2% scaled carps in lake Alaotra (n=44), 89.0% in the Tsiribihina drainage basin (n=136), 40.0% in the Betsiboka drainage basin (n=25) and 13.3% in the Mangoky drainage basin (n=75). In farms, there was little variation between regions, with 15.0 to 17.5% scaled carps in the farms from the three drainage basins sampled.

Genetic and phenotypic diversity of Malagasy common carp for scale cover

Among the 236 individual samples sequenced for the S/s point mutation of *fgfr1a1*,suprisingly the whole set of 103 feral individuals with a fully-scaled phenotype were found homozygous s/s (Table 1), showing that a full scale cover can be achieved even though these fish carry a loss-of-function mutation, which normally implies the mirror (incomplete) phenotype. As expected, the 89 mirror carps analyzed also showed this homozygous s/s genotype. Scaled carps sampled from fish-farms predominantly (35/44) carried at least one S (wild-type) allele nucleotide at the investigated locus.

The 236 Malagasy carps sequenced for *fgfr1a1* were also genotyped for 9 microsatellite markers, together with 72 Hungarian carps representative of the strains introduced in 1979. Clustering analysis[37,38]showed that two clusters were present among these fish.

Fully-scaled carps with mirror (s/s) genotype almost exclusively belonged to the first cluster, while regular scaled carps (S/s or S/S genotype) were all belonging to the second cluster (Figure 1a). All Hungarian carps, irrespective of their scale cover, belonged to the second cluster, which we then qualify as “Hungarian farmed”. Together with the history of introductions, and the fact that fully-scaled carps were observed in the wild before the second introduction from France in 1959, this suggests that feral carps (mostly fully-scaled) derive from the first introduction of carps in 1912. We refer to this first cluster as the “pioneer” cluster. Mirror carps could belong to any of both clusters. Most fish from the wild belonged to the pioneer cluster, with the notable exception of 22 fish caught from the Mandarano River, in the Mangoky drainage basin (Figure 1b). The mean allelic richness was greater in Malagasy

cultured populations ($A_{R\ mean}$ = 7.8) than in feral populations ($A_{R\ mean}$ = 5.2) suggesting a higher genetic diversity in cultured populations. Analysis of private alleles showed only three alleles specific to feral populations (all with frequency <5%) vs. 26 (of which 7 had a frequency >5%) alleles which were present only in cultured populations. Twelve of those 26 private alleles (including 5 of the 7 most frequent ones) were found in the Hungarian samples. This suggests that the greater allelic richness of cultured populations is partly due to introgression of Hungarian genes, but that there is a low gene flow from cultured to feral populations. The population from Mandarano river that showed introgression from cluster 2 (Figure 1) was also shown to be intermediate between Hungarian, Malagasy farmed and feral populations, suggesting a possible effect of restocking in this area (see figure S2).

The pictures of 209 out of the 236 carps allowed to quantify the number of scales on one flank. Scales were counted on 105 scaled carps which were homozygous for the loss-of-function mutation (s/s genotype), 76 carps exhibiting a mirror phenotype (also with s/s genotype) and 27 scaled carps carrying the functional S/s or S/S genotype. Number of scales had a bimodal distribution (Fig. 2). The lower mode corresponded to fish carrying an s/s (loss of function) genotype, while the upper mode corresponded to fish carrying at least one functional allele (S/S or S/s). The 27 S/S or S/s scaled carps had between 300 and 448 scales (383 on average), which was consistently greater than the scale number of the 105 homozygous s/s fully-scaled carps (90-280 scales, 161 on average). This highlights that the mechanism leading to full scale cover is not the same in S-carrying carps and s/s homozygotes. The 181 s/s carps, including mirror and fully-scaled ones, displayed a wide range of scale covers, with a number of scales varying from 55 to 280 scales. This shows that both for carps carrying one functional *fgfr1a1* allele and for carps homozygous for the loss-of-function mutation, the number of scales presents significant phenotypic variation (Fig. 2).

Heritability of scale cover

Scales were counted on 196 offspring from a factorial crossing of 4 s/s males (2 fully-scaled, 2 with mirror phenotype) with 4 s/s females (all mirror). The scale counts of the males ranged from 100 to 206, and those of the females from 87 to 123. The offspring had 96.9 scales on average (range 34-197). The effect of the male parent on scale number was highly significant ($F_{3,178}$=12.37, P<0.0001), that of the female parent was also significant ($F_{3,178}$=3.09, P=0.03) while their interaction was not ($F_{9,178}$=1.77, P=0.08). The distributions of scale counts in the

offspring of the 4 males largely overlapped with each other (figure S3). Only male FSM2, which had the highest scale number (n=206), produced some fully-scaled offspring (N=12 out of 68, having between 122 and 197 scales). This shows that that there is no secondary dominant locus substituting *fgfr1a1* in fully-scaled s/s fish. The fully-scaled offspring of male FSM2 were observed in its crosses with females M2 (5/15), M3 (5/15) and M4 (2/28), but not M1 (0/10). If full scale cover in s/s fish was governed by a recessive compensatory mutation, this would mean that females M2 to M4 carry it and that male FSM2 is homozygous. This would lead to an expectation of 50% fully scaled offspring in these crosses, but this was not observed (χ^2=22.3, 1 d.f., $P<10^{-5}$). This would also imply the presence of fully-scaled fish in the offspring of male FSM1, and this was also not observed. Using the same data, heritability of scale number was estimated to be 0.49±0.16, showing that this trait is under strong quantitative genetic control in s/s individuals. The distribution of the number of scales could be considered normal in the offspring of two dams and three sires (Shapiro-Wilk test, P>0.05), while it significantly departed from normality for two (mirror) dams (Dam M2, P<0.01; Dam M4, P<0.04) and one fully-scaled sire (Sire FSM2, P<0.02). All distributions were however unimodal (Dip test[48], P>0.70 – see Figure S3), so that there was no apparent segregation of a major gene for scale cover in the s/s progenies tested, contrary to what was observed when s/s and S-carrying fish were compared (figure 2). In light of these results, we conclude that preexisting polygenic variation was selected and compensated for the absence of any functional version of the major gene *fgfr1a1* in Malagasy feral carp populations.

Effect of scale cover on survival

The survival from the larval stage to 109 days post-fertilization was 51.8% in a pond where offspring from S/s males mated with s/s females were stocked. Out of the 312 offspring assigned to their parents, 169 were scaled and 143 mirror. These numbers did not significantly depart from the 1:1 ratio expected under equal survival and Mendelian segregation of S (χ^2=2.17, 1 *d.f.*, *P*>0.14). When differential survival was evaluated until the age of 11 months in 6 rice fields and 4 ponds, the average survival was 84.8% in ponds and 28.0% in rice fields, but in all rearing units except one rice field where scaled carp survival was higher (χ^2=3.846, 1 *d.f.*, *P*=0.049), survival was similar between scaled and mirror carps (P>0.6).

Pace of evolution

We calculated the rate of evolution of scale cover considering that scale counts in contemporary mirror carps (mean=94.6, SD=22.7) were representative of the carps introduced

in 1912. The present scale count in fully-scaled mirror carps (mean=161.0 SD=36.9) was supposed to be reached in 100 years (1912-2012 - 40 generations), giving estimates of 5.3 KDar or 0.056 haldanes, which is in the 3% highest rates of genetic change[49], implying a strong natural selection intensity.

Discussion

In the present study, we examined the present phenotypic and genotypic status of common carp in Madagascar, following an introduction in 1912 of fish homozygous for a loss-of-function mutation in *fgfr1a1* causing a reduction in scale cover, *i.e.* the mirror phenotype.

We showed that feral carps were predominantly fully scaled, while farmed ones were mostly mirror, although some fish with full scale cover could also be found in farms. However, in farms, most scaled fish carried a functional S allele for *fgfr1a1*, which presumably originated from Hungarian carps introduced in 1979, or from later introductions. Most of the feral fish belonged to the same "pioneer" cluster, and some fish from this cluster were also present in farmed populations. The reappearance of the wild-type-like scaled phenotype was first described in 1958[25]. At that time it was mostly recorded in the lowlands of the west (>95% scaled carps) while in lake Alaotra the percentage of scaled carps was 85%, and only 70% in the highlands[27]. This trend was also found in the present study, with the exception of the Mangoky drainage basin where the proportion of scaled carps was only 13.3%. The first remarkable finding of the present study is that all scaled carps from the wild are still homozygous for the loss-of-function mutation of *fgfr1a1*, like their mirror ancestors. Hence, despite the mutation, they were able to evolve a compensatory mechanism to produce a full scale cover. This also indirectly confirms that the carps introduced in 1912 (a time where the genetic basis of the mirror phenotype was unknown) were true mirrors, homozygous for the loss-of-function allele.

We could not show any effect of scale cover on survival in the environment tested, but previous data show that scaled carps have a higher survival than mirror carps[18], e.g. with a higher resistance to parasitic infections[50]. An interesting parallel example is that of the US, where both mirror (N=227) and scaled (N=118) carps were introduced in 1887, and where scaled carps now represent >98% of the wild individuals[51]. In the US case however, the scaled fish introduced seemingly possessed the functional *fgfr1a1* allele, as first generation offspring of the introduced broodstock were nearly all fully scaled[51]. In the case of Madagascar, regular scaled (SSnn) fish were introduced in 1979, but we did not find them in

the wild. Even though, despite escapes from farms cannot be avoided, and restocking operations with farmed fish are common, the lack of any wild SSnn scaled carp here suggests that they do not have a decisive competitive advantage with the ssnn "neoscaled" fish. Interestingly, irrespective of scale cover, fish from the "Hungarian farmed" cluster were not found in the wild, except in the Mangoky drainage basin, which is specific in the fact that scaled fish are rare (13.3%), even among fish from the "pioneer" cluster. Taken together, this indicates that natural selection against incomplete scale cover must be high in most (maybe not all) natural environments, precluding gene flow from farms to the wild. The nature of the selecting agent(s) however remains unknown. The inferred evolutionary rate for scale number of 5.3 KDar or 0.056 haldanes is high[47,49], but subject to caution for two reasons: first, we do not know how many scales the mirror fish introduced in 1912 carried, and the possible presence of fully-scaled mirror fish in this initial stock (or further undocumented introductions) cannot be excluded with certainty, although fully-scaled mirror carps are not normally seen in European farmed carp populations. Second, the present predominance of mirror fish in farms, and their relatively low average scale number may also be the result of introductions of new mirror fish in 1959, 1979 or later. Additionally negative selection for scale number by fish farmers is likely to happen, as scales are seen as a complication for cooking. Conversely, as a majority of scaled fish was already present in the wild at the end of the 1950s, the time for selection to operate was probably shorter than what we estimated.

Although we cannot totally exclude an input from environmental effects on the observed scale cover patterns, our results clearly support a polygenic control of the number of scales in Malagasy feral carps. First, phenotypic plasticity for scale number would not lead to the observed very low level of gene flow from farms to the wild, this being especially true in zones where fully-scaled feral carps are frequent or dominate (all except the Mangoky drainage basin). In addition, our controlled breeding assay showed a significant heritable component of scale number in s/s fish (h^2= 0.49), and the absence of a simple Mendelian system underlying this variation. The parental scale numbers were shown to significantly influence the phenotypes in the F1 individuals, which also displayed a large variance in scale number. For example, the progeny from male M1 (100 scales) exhibited from 34 to 143 scales, whereas from 63 to 197 scales were identified in the progeny of male FSM2 (206 scales). Such a pattern suggests that the number of scales is likely to be affected by several genes, as suggested earlier [19,20], not precluding the possibility that this variation may be governed by a few major QTLs as seen in lateral plate number variation in the threespine

stickleback[12]. Indeed, though the Mendelian S/s system still explains most of the variation in farmed populations (see Table 1), polygenic (or at least oligogenic) genetic determinism would fit well with the recent hypothesis that some variation in scale phenotypes of common carp or zebrafish would be caused by dose-dependent signaling[20,21]. Overall, the most likely explanation for the rapid evolution of scale cover in Madagascar feral carps is natural selection of pre-existing polygenic variation, uncovered by the homozygous s/s genotype of *fgfr1a1* in the carps introduced in 1912. Genetic variation for scale cover may exist in wild type (S-carrying) fully-scaled carp (for which we showed at least phenotypic variation in scale numbers – fig.2), but have little effect on fitness in such fully-scaled carps – and in this sense remain cryptic and unselected[10]

Studies performed on a range of laboratory models have addressed the topic of compensatory evolution in response to gene loss. Mutated populations were shown to evolve towards better fitness without necessarily resorting to molecular convergence, even when the wild-like phenotype was restored. Instead, compensatory mechanisms predominantly relied on alternative pathways involving several genes[6,7] and resulted in strong fitness benefits that could arise very rapidly[9]. Similarities with such experimental settings and outcomes can be found in the history of the common carp in Madagascar. The initial introduction of a population that remained shielded from migration at least during the first generations, and that was fixed for a mutation affecting fitness, resulted in a rapid adaptation to Malagasy waters. Under the adaptive landscape concept, the pioneer mirror carp population was possibly located in a fitness valley and subsequent generations climbed an alternative route through standing genetic variation to gain fitness. Finally, this serendipitous experiment on carp illustrates in nature the observations previously reported from laboratory evolution and highlights the potential of "unplanned natural" experiments to help better understand rapid evolution in an ecological context[15].

The present work brings evidence for a rescue of the wild-type-like scale cover through the likely selection of polygenes from standing genetic variation. This provides a visible and striking example that evolutionary convergence (*i.e.* to wild-type-like scale cover) can use other routes than reversion mutation, and suggests that natural populations can host enough capacities for adaptation on the short-term to face a sudden environmental change, even if a harmful mutation was formerly fixed.

Ethics

Experiments with live fish were performed under the responsibility of Dr Marc Vandeputte (authorization B-34-437 to perform animal experiments on fish), in accordance with European standards. No specific authorization was required for this project according to French and Malagasy law.

Data Accessibility

The datasets supporting this article have been uploaded as part of the supplementary material.

Competing interests

We have no competing interests

Authors contributions

M.V. and R.G. conceived, designed and supervised the study. R.G., M.R., Z.J. coordinated field sample collection. J.N.H., C.H., A.V., M.R., M.V. performed experiments. J.N.H, F.A., M.R., M.V. analysed data and drafted the manuscript. All authors approved the final version.

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References

1. Teotónio, H. & Rose, M. R. 2001 Perspective: reverse evolution. *Evolution (N. Y).* **55**, 653. (doi:10.1554/0014-3820(2001)055[0653:PRE]2.0.CO;2)
2. Lande, R. 1994 Risk of population extinction from fixation of new deleterious mutations. *Evolution (N. Y).* **48**, 1460–1469.
3. Bull, J. J., Badgett, M. R., Wichman, H. A., Huelsenbeck, J. P., Hillis, D. M., Gulati, A., Ho, C. & Molineux, I. J. 1997 Exceptional convergent evolution in a virus. *Genetics* **147**, 1497–1507.
4. Moore, F. B.-G., Rozen, D. E. & Lenski, R. E. 2000 Pervasive compensatory adaptation in Escherichia coli. *Proc. R. Soc. London B Biol. Sci.* **267**, 515–522.
5. Levin, B. R., Perrot, V. & Walker, N. 2000 Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics* **154**, 985–997.
6. Harcombe, W. R., Springman, R. & Bull, J. J. 2009 Compensatory evolution for a gene deletion is not limited to its immediate functional network. *BMC Evol. Biol.* **9**, 106. (doi:10.1186/1471-2148-9-106)
7. Szamecz, B. et al. 2014 The genomic landscape of compensatory evolution. *PLoS Biol.* **12**. (doi:10.1371/journal.pbio.1001935)
8. Estes, S. & Lynch, M. 2003 Rapid fitness recovery in mutationally degraded lines of Caenorhabditis elegans. *Evolution* **57**, 1022–1030.
9. Chandler, C. H., Chadderdon, G. E., Phillips, P. C., Dworkin, I. & Janzen, F. J. 2012 Experimental evolution of the Caenorahabditis elegans sex determination pathway. *Evolution (N. Y).* **66**, 82–93.
10. Rajon, E. & Masel, J. 2013 Compensatory evolution and the origins of innovations. *Genetics* **193**, 1209–1220. (doi:10.1534/genetics.112.148627)
11. Wiser, M. J., Ribeck, N. & Lenski, R. E. 2013 Long-term dynamics of adaptation in asexual populations. *Science (80-.).* **342**, 1364–1367. (doi:10.1126/science.1243357)
12. Colosimo, P. F., Peichel, C. L., Nereng, K., Blackman, B. K., Shapiro, M. D., Schluter, D. & Kingsley, D. M. 2004 The genetic architecture of parallel armor plate reduction in threespine sticklebacks. *PLoS Biol.* **2**, 635–641.
13. Reznick, D. N., Shaw, F. ., Rodd, F. H. & Shaw, R. G. 1997 Evaluation of the rate of evolution in natural populations of guppies (Poecilia reticulata). *Science (80-.).* **275**, 1934–1937. (doi:10.1126/science.275.5308.1934)
14. Steiner, C. C., Weber, J. N. & Hoekstra, H. E. 2007 Adaptive variation in beach mice produced by two interacting pigmentation genes. *PLoS Biol* **5**, e219.
15. Irschick, D. J. & Reznick, D. 2009 Field experiments, introductions, and experimental evolution. In *Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments* (eds T. Garland Jr. & M. R. Rose), pp. 173–194. University of California Press. (doi:10.1525/california/9780520247666.003.0008)
16. Balon, E. K. 2004 About the oldest domesticates among fishes. *J. Fish Biol.* **65**, 1–27. (doi:10.1111/j.1095-8649.2004.00563.x)
17. Casal, C. 2006 Global documentation of fish introductions: the growing crisis and recommendations for action. *Biol. Invasions* **8**, 3–11. (doi:10.1007/s10530-005-0231-3)

18. Kirpichnikov, V. S. 1999 Genetics and breeding of common carp. *INRA Ed. Paris* , 97.

19. Rohner, N., Bercsényi, M., Orbán, L., Kolanczyk, M. E., Linke, D., Brand, M., Nüsslein-Volhard, C. & Harris, M. P. 2009 Duplication of fgfr1 permits Fgf signaling to serve as a target for selection during domestication. *Curr. Biol.* **19**, 1642–1647. (doi:10.1016/j.cub.2009.07.065)

20. Casas, L., Szűcs, R., Vij, S., Goh, C. H., Kathiresan, P., Németh, S., Jeney, Z., Bercsényi, M. & Orbán, L. 2013 Disappearing scales in carps: re-visiting Kirpichnikov's model on the genetics of scale pattern formation. *PLoS One* **8**, e83327. (doi:10.1371/journal.pone.0083327)

21. Harris, M. P., Rohner, N., Schwarz, H., Perathoner, S., Konstantinidis, P. & Nüsslein-Volhard, C. 2008 Zebrafish eda and edar mutants reveal conserved and ancestral roles of ectodysplasin signaling in vertebrates. *PLoS Genet.* **4**, e1000206. (doi:10.1371/journal.pgen.1000206)

22. Colosimo, P. F. et al. 2005 Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science (80-.).* **307**, 1928–1933.

23. Knecht, A. K., Hosemann, K. E. & Kingsley, D. M. 2007 Constraints on utilization of the EDA-signaling pathway in threespine stickleback evolution. *Evol. Dev.* **9**, 141–154. (doi:10.1111/j.1525-142X.2007.00145.x)

24. O'brown, N. M., Summers, B. R., Jones, F. C., Brady, S. D. & Kingsley, D. M. 2015 A recurrent regulatory change underlying altered expression and Wnt response of the stickleback armor plates gene EDA. *Elife* **2015**, 1–17. (doi:10.7554/eLife.05290)

25. Kiener, A. 1958 Intérêt et perspectives de la pisciculture de la carpe à Madagascar. *Bull Madagascar* **8**, 693–702.

26. Lemasson, L. 1957 Réflexions sur la pêche et la pici a Madagascar.pdf. *Bois Forêts des Trop.* **52**, 57–61.

27. Kiener, A. 1963 *Poissons, pêche et pisciculture à Madagascar*. Publicatio. Nogent sur Marne, France: Editions du CTFT.

28. Moreau, J., Arrignon, J. & Jubb, R. A. 1988 Les introductions d'espèces étrangères dans les eaux continentales africaines : intérêt et limites. In *Biologie et écologie des poissons d'eau douce africains* (eds C. Lévèque & M. N. Bruton), pp. 329–425. Paris, France.

29. Láng, W. M. 1980 Transport of fingerlings to Africa. *Halászat* **XXVI**, 43.

30. Weckx, S., Del-Favero, J., Rademakers, R., Claes, L., Cruts, M., De Jonghe, P., Van Broeckhoven, C. & De Rijk, P. 2005 novoSNP, a novel computational tool for sequence variation discovery. *Genome Res.* **15**, 436–442. (doi:10.1101/gr.2754005)

31. Zheng, X., Kuang, Y., Zhang, X., Lu, C., Cao, D., Li, C. & Sun, X. 2011 A genetic linkage map and comparative genome analysis of common carp (Cyprinus carpio L.) using microsatellites and SNPs. *Mol. Genet. Genomics* **286**, 261–277. (doi:10.1007/s00438-011-0644-x)

32. Yue, G. H. & Orban, L. 2002 Polymorphic microsatellites from silver crucian carp (Carassius auratus gibelio Bloch) and cross-amplification in common carp (Cyprinus carpio L.). *Mol. Ecol. Notes* **2**, 534–536. (doi:10.1046/j.1471-8286.2002.00308.x)

33. Crooijmans, R. P. M. a, Poel, J. J. Van Der, Groenen, M. a M., Bierbooms, V. a F. & Komen, J. 1997 Microsatellite markers in common carp (Cyprinus carpio L.). *Anim. Genet.* **28**, 129–134. (doi:10.1111/j.1365-2052.1997.00097.x)

34. Weir, B. S. & Cockerham, C. C. 1984 Estimating F-Statistics for the Analysis of Population Structure. *Evolution (N. Y).* **38**, 1358–1370. (doi:10.2307/2408641)

35. Goudet, J. 1995 Fstat (version 1.2): a computer program to calculate F-statistics. *J. Hered.* **86**, 485–486. (doi:10.1093/jhered/esg066)

36. Rice, W. R. 1989 Analyzing Tables of Statistical Tests. *Evolution (N. Y).* **43**, 223–225. (doi:10.2307/2409177)

37. Pritchard, J. K., Stephens, M. & Donnelly, P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

38. Evanno, G., Regnaut, S. & Goudet, J. 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620. (doi:10.1111/j.1365-294X.2005.02553.x)

39. Sokal, R. R. & Michener, C. D. 1958 A statistical method for evaluating systematic relationships. *Univ. Kans. Sci. Bull.* **38**, 1409–1438.

40. Perrier, X. & Jacquemoud-Collet, J. P. 2006 DARwin software. http://darwin.cirad.fr.

41. Horvath, L., Szabo, T. & Burke, J. 1997 Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species. *Pol. Arch. Hydrobiol.* **44**, 221–226.

42. Wang, D., Liao, X., Cheng, L., Yu, X. & Tong, J. 2007 Development of novel EST-SSR markers in common carp by data mining from public EST sequences. *Aquaculture* **271**, 558–574.

43. David, L., Rajasekaran, P., Fang, J., Hillel, J. & Lavi, U. 2001 Polymorphism in ornamental and common carp strains (Cyprinus carpio L.) as revealed by AFLP analysis and a new set of microsatellite markers. *Mol. Genet. Genomics* **266**, 353–362.

44. Vandeputte, M., Mauger, S. & Dupont-Nivet, M. 2006 An evaluation of allowing for mismatches as a way to manage genotyping errors in parentage assignment by exclusion. *Mol. Ecol. Notes* **6**, 265–267.

45. Abràmofff, M. D., Magalhães, P. J. & Ram, S. J. 2004 Image processing with ImageJ. *Biophotonics Int.* **11**, 36–42. (doi:10.1117/1.3589100)

46. Groeneveld, E., Kovac, M. & Mielenz, N. 2008 *VCE user's guide and reference manual version 6.0.* Neustadt, Germany: Friedrich Loeffler Institute.

47. Kinnison, M. & Hendry, A. 2001 The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* **112-113**, 145–164. (doi:10.1023/A:1013375419520)

48. Hartigan, J. & Hartigan, P. 1985 The dip test of unimodality. *Ann. Stat.* **13**, 70–84.

49. Bell, G. 2008 *Selection: the mechanism of evolution*. 2nd edn. Oxford, UK: Oxford University Press.

50. Price, D. J. & Clayton, G. M. 1999 Genotype-environment interactions in the susceptibility of the common carp, Cyprinus carpio, to Ichthyophthirius multifiliis infections. *Aquaculture* **173**, 149–160.

51. Walden, H. T. 1964 *Familiar freshwater fishes of America*. New York: Harper & Row.

Figure and Tables captions

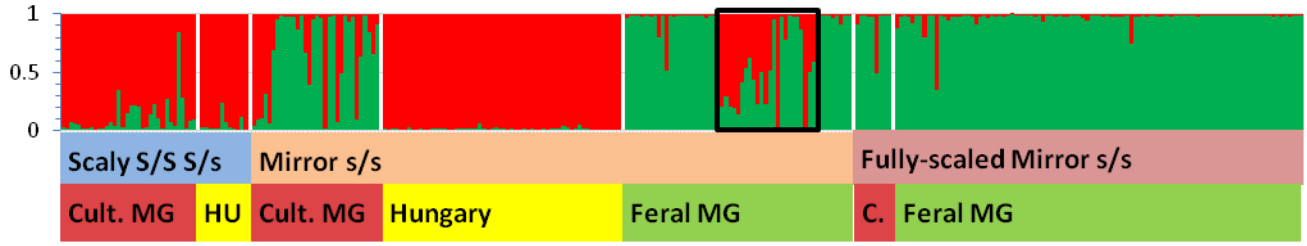
Table 1 Type of scale cover – fully scaled or incomplete – as a function of their *fgfr1a1* genotype at the polymorphic loss-of-function point mutation site[19] in 236 carps sampled from different regions of Madagascar.

Figure 1: Population clusters inferred by STRUCTURE[37] from genotypes at 9 microsatellite markers in 236 feral and cultured Malagasy carps sampled in 2012, and in 72 samples from Hungary. Individuals were ordered according to either a) their scale cover phenotype and their genotype for the loss of function mutation of *fgfr1a1*, or b) to the sampling region. Cluster 1 (pioneer) in green, cluster 2 (Hungarian farmed) in red. Samples from Mandarano river in a black frame.

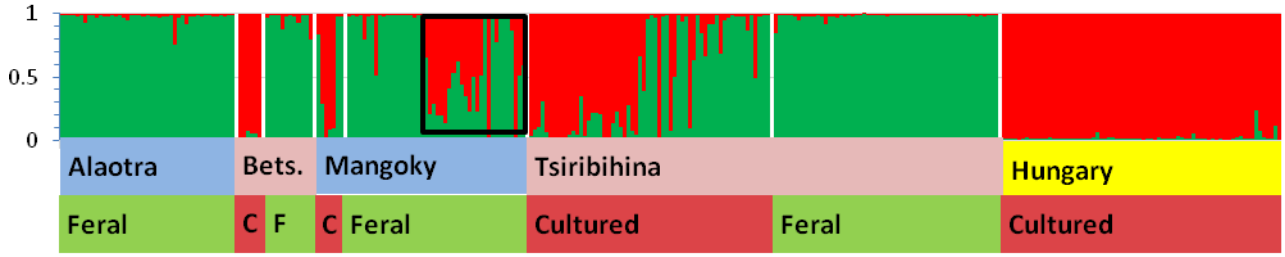
Figure 2: Left panel: Distribution of the number of scales quantified on one flank for 208 carps which were genotyped for the loss-of-function mutation in *fgfr1a1*. Colors indicate the type of scale cover followed by *fgfr1a1* genotype. Right panel: Phenotype (M: Mirror; FSM: Fully-Scaled Mirror; S: Scaled) and genotype for *fgfr1a1* for 6 carps representative of the distribution of scale numbers.

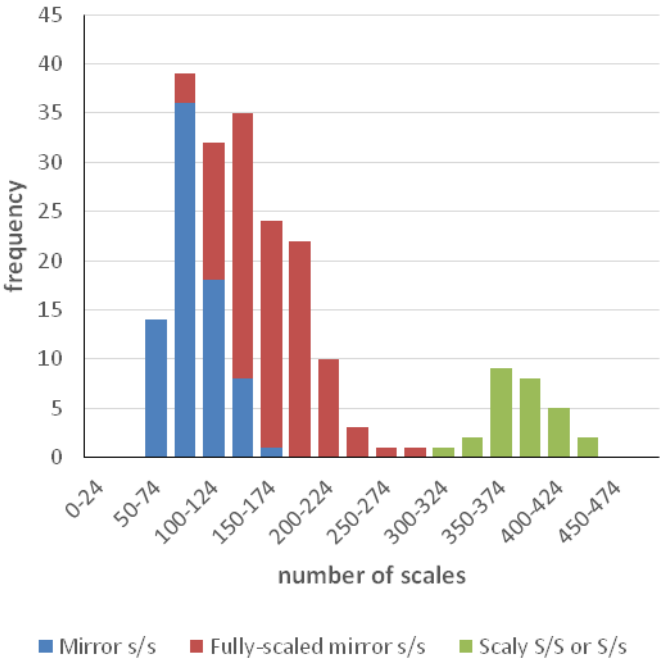
Phenotype		*Fgfr1a1* Genotype		
		S/S	S/s	s/s
Fully-scaled	Farmed	5	30	9
	Feral	0	0	103
Incomplete	Farmed	0	0	32
	Feral	0	0	57

a)


1
0.5
0
Scaly S/S S/s
Mirror s/s
Fully-scaled Mirror s/s
Cult. MG
HU
Cult. MG
Hungary
Feral MG
C.
Feral MG

b)


1
0.5
0
Alaotra
Bets.
Mangoky
Tsiribihina
Hungary
Feral
C
F
C
Feral
Cultured
Feral
Cultured



Pheno	Geno	N scales	
M	s/s	65	
M	s/s	82	
FSM	s/s	118	
FSM	s/s	184	
FSM	s/s	263	
S	S/s	393