Comparison of rearing performances and intermuscular bone number in the mirror and nude genotypes of common carp (Cyprinus carpio L.) in a controlled field test in Madagascar

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A B S T R A C T

Nude carp, a genotype of common carp which are devoid of scales, have been banned from farmed populations of carp in Europe due to both the lethal effect of the N (nude) gene when in homozygous state, and to the negative pleiotropic effects on growth and survival (especially in harsh conditions) of this same gene in the heterozygous state, which produces the nude phenotype. In Madagascar, where climatic variations are less extreme than in Eastern Europe, the nude phenotype is valued both by farmers and consumers, for its good growth and supposed low number of intermuscular bones. We performed an on-farm experiment using a "common garden" design to control environmental variation, in order to compare the growth and survival, as well as the number of intermuscular bones of nude carp to other common scale cover phenotypes of the same species, the mirror and scaly carps. We found that survival of nude carps was lower or equal to that of mirror carps at all stages of the farming process, while growth performance was lower than that of mirror carps in some ponds only. Globally, the biomass production per fish stocked was always lower in nude carp compared to mirror carp. The number of intermuscular bones was the same in nude, mirror and scaly carps. We conclude that as in Europe, it would be valuable to farm mirror rather than nude carps, as the supposed benefits of the latter are not supported by our experiment in typical Malagasy farming conditions.

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1. Introduction

Cyprinids, and among them carps, are the most farmed fish species in the world, as they accounted for 70% of the world production of fish in 2010 (FAO, 2012). Common carp is the main fish species farmed in Madagascar, where it was introduced in 1912 (Kiener, 1963) and accounts for 74% of the total aquaculture production of the country (2600 t of a total of 3507 t FAO, 2015). Carp is farmed in rural areas, mainly in rice-field cultures but also in ponds (Brugère, 2006) and is important for food security in those areas. After a steady increase in the 1990’s, the production is now stagnating, and special attention to the genetic quality of farmed carp is warranted. One of the striking features of common carp farmed populations is the presence of four scale cover phenotypes, which were shown to be governed by the combination of two bi-allelic (S/s = scaly, N/n = nude) Mendelian loci in the 1930–1940’s (reviewed by Kirpichnikov, 1999). Wild-type scaly carp have the Ssnn or Ssnn genotype, while mirror carps, with a few scattered large scales, have the ssnn genotype (Fig. 1). The presence of the heterozygous N allele turns scaly carps to linear carps (Fig. 1: genotypes SSNn or SsNn), with a few lines of large scales, while it turns mirror carps into nude carps (ssNn), with (almost) no scales (Fig. 1). When the N gene is in homozygous state, it is lethal and induces mortality at the stage of hatching (Wohlfarth et al., 1963). Together with the inferior performance of the N-carrying genotypes, especially in harsh conditions, in terms of growth and survival (Kirpichnikov, 1999), this led to the voluntary elimination of nude and linear carps from the main farmed strains of common carp in Europe (Hollebecq and Haffray, 1994). However, in Madagascar, where mild climatic conditions (mean monthly temperatures ranging from 14 to 28 °C) are favorable to the development and growth of carp, nude carps are still rather commonly farmed, and are valued both by farmers and consumers. Farmers generally consider that they have equiv-
alent or even better growth and survival than mirror carps. On the consumer side, nude carps have a reputation to be easier to prepare due to the absence of scales, but also – although this is not proven – to have less intermuscular bones than mirror or scaly carps – intermuscular bones being a serious limitation for the development of carp consumption. These supposed qualities of nude carps (growth, survival, less intermuscular bones) give a premium to the farming of nude carps. As the inferior performance of nude carps for growth and survival was essentially demonstrated in Europe and in harsh conditions (Kirpichnikov, 1999; Steffens, 1975), it is worthy to examine its reality in very different conditions. The statement by local farmers that nude carps have less intermuscular bones may seem strange, but although the genes underlying the nude phenotype have not been identified to date (Casas et al., 2013), the nude phenotype is known to imply pleiotropic effects of reduction of bony structures like fin rays and pharyngeal teeth (Casas et al., 2013; Kirpichnikov, 1999; Steffens, 1975). To our knowledge, there are no studies comparing intermuscular bones number between nude carps and other genotypes, but recent results show less intermuscular bones in a mirror strain than in a scaly strain of common carp in China (Cao et al., 2015). Moreover, recent investigations in the grass carp Ctenopharyngodon idella show that mutations provoking scale reduction can also result in intermuscular bone deficiency (Xu et al., 2015). Therefore, the effect of the N gene on intermuscular bone number is also worth investigating.

In this context, we set up a controlled experiment in a “common garden” setting, in field conditions, to evaluate in typical Malagasy carp farming conditions (1) the growth and survival of nude carps compared to mirror carps and (2) the variations of intermuscular bones number in nude, mirror and scaly carps.

### 2. Material and methods

#### 2.1. Production of test progenies

Artificial reproduction was performed in the Centre Régional de Formation Professionnelle Agricole (Antanentimoboa hangy, Analavory, Itasy, Madagascar). We performed a crossing between nude sires (ssNn) and mirror dams (ssnn), as in such a cross the N gene will segregate, producing 50% ssNn (nude) and 50% ssnn (mirror) offspring on the same genetic background. Three nude males from a farm in the Vakinankaratra region were mated with 7 mirror females from a farm in the Itasy region. For inducing ovulation in females Ovopel (D-Ala6, Pro9-Net-mGnRH, Unic-trade, Hungary) was homogenized using 1 pellet/ml in 0.9% NaCl solution (Horvath et al., 1997), 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 h later. Spawning occurred 12 h after the second injection, and the spawns of the 7 females were stripped and mixed to produce a pool of eggs. The sperm of the 3 males had been collected 12 h in advance by stripping, and was stored at 4 °C in 5 ml syringes (max 1 ml sperm/syringe).

A total of 147 g of eggs from the pool was split in three equal parts of 49 g, each being gently mixed with 0.5 ml sperm from one male, and activated with 50 ml of activation solution (3 g/lurea, 4 g/l NaCl). One minute after activation, all three fertilization batches were mixed in a plastic bucket where they were 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 incubated in a McDonald jar at an average temperature of 24 °C. Hatching occurred at 47 h post-fertilization, and larvae were transferred to a resorption tank with flow-through water.

#### 2.2. Rearing of progenies and phenotyping

Two batches of 1300 larvae were counted by volumetry, and placed at 2 days post-hatching in two 0.15 m³ hapas, where they were fed 3 times/day a filtered suspension of egg yolk, wheat flour, dried blood and soybean meal. At 12 days post-hatching, all larvae from the first hapa were transferred to a 100 m² pond (pond P) in Antanetimoboa hangy, while the larvae from the second hapa were split in two approximately equal batches and transferred to two 25 m² ponds (L1 and L2) in Analavory. In addition to the natural feed from the pond, supplemental feed was given twice a week and consisted of 50% maize flour, 20% cassava meal, 15% peanut oilcake and 15% dried blood. Juveniles were harvested at 108 days post-hatching in pond P and at 110 days post-hatching in ponds L1 and L2, and were recorded for live body weight and classified for scale cover type. In both sites, the average pond temperature during the period was 23 °C.

For ongrowing, 4 batches of 50 randomly selected mirror and 50 randomly selected nude carps were produced from pre-growing pond P. All fish were individually weighed prior to stocking. Two of the batches were kept in the same farm at Ananetoimboahangy in two ponds of 100 m² (P1 and P2) while the other two batches were transferred to another farm in Miarnarivo where they were grown in rice fields of 100 m² (R1 and R2). A third batch (MNS) was formed with 70 mirror, 70 nude and 70 scaly carps, the latter originating from the crossing of heterozygous scaly (Ssnn) sires on the same set of dams on the same day for another experiment. We selected the pond and the scaly juveniles from this second experiment in order to have progenies size-matched to our mirror and nude progenies. All fish were individually weighed prior to stocking. This MNS batch was reared in a 1000 m² pond in Miarnarivo, for further intermuscular bones counting. In all sites, natural feed from the pond was supplemented twice a week with a mix of 60% maize flour, 20% cassava meal and 20% peanut oil cake.

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**Fig. 1.** The four scale cover phenotypes of carp, as exemplified by juvenile common carps from Madagascar (a) scaly (b) mirror (c) linear (d) nude.
Table 1
Relative performance of mirror and nude carps from two nursery ponds. Initial number is based on the 1:1 segregation of the mirror and nude genotypes, as it could not be recorded in the seeded larvae.

<table>
<thead>
<tr>
<th>Pond</th>
<th>Scale cover phenotype</th>
<th>Initial number of larvae</th>
<th>Final number</th>
<th>survival</th>
<th>Mean BW (g)</th>
<th>Final biomass (g)</th>
<th>Biomass/seeded larva (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>Mirror</td>
<td>650</td>
<td>249</td>
<td>38.3%a</td>
<td>3.57h</td>
<td>889</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>Nude</td>
<td>650</td>
<td>187</td>
<td>28.8%a</td>
<td>2.81h</td>
<td>525</td>
<td>0.81</td>
</tr>
<tr>
<td>L1</td>
<td>Mirror</td>
<td>325</td>
<td>93</td>
<td>28.6%a</td>
<td>3.90h</td>
<td>363</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>Nude</td>
<td>325</td>
<td>82</td>
<td>25.2%a</td>
<td>3.60h</td>
<td>295</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Table 2
Performance of carp of different scale cover phenotypes during 5 months of grow-out in two rice fields (R1, R2) and two ponds (P2, MNS). Within ponds, survival and final body weight data with different superscripts are significantly different (P < 0.05).

<table>
<thead>
<tr>
<th>Pond</th>
<th>Scale cover phenotype</th>
<th>Initial number</th>
<th>Final number</th>
<th>survival</th>
<th>Initial BW (g)</th>
<th>Final BW (g)</th>
<th>Biomass gain per juvenile(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Mirror</td>
<td>50</td>
<td>20</td>
<td>40%a</td>
<td>4.21</td>
<td>53.0a</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>Nude</td>
<td>50</td>
<td>18</td>
<td>36%a</td>
<td>3.08</td>
<td>39.2h</td>
<td>11.0</td>
</tr>
<tr>
<td>R2</td>
<td>Mirror</td>
<td>50</td>
<td>42</td>
<td>84%a</td>
<td>4.44</td>
<td>39.1h</td>
<td>28.4</td>
</tr>
<tr>
<td></td>
<td>Nude</td>
<td>50</td>
<td>21</td>
<td>42%a</td>
<td>3.27</td>
<td>37.5h</td>
<td>12.5</td>
</tr>
<tr>
<td>P2</td>
<td>Mirror</td>
<td>50</td>
<td>13</td>
<td>26%a</td>
<td>3.10</td>
<td>74.7h</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>Nude</td>
<td>50</td>
<td>10</td>
<td>20%a</td>
<td>2.79</td>
<td>79.0h</td>
<td>13.0</td>
</tr>
<tr>
<td>MNS</td>
<td>Mirror</td>
<td>70</td>
<td>69</td>
<td>99%a</td>
<td>4.48</td>
<td>45.6e</td>
<td>40.5</td>
</tr>
<tr>
<td></td>
<td>Nude</td>
<td>70</td>
<td>15</td>
<td>21%c</td>
<td>4.67</td>
<td>51.1e</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>Scaly</td>
<td>70</td>
<td>47</td>
<td>67%b</td>
<td>5.62</td>
<td>53.4e</td>
<td>30.2</td>
</tr>
</tbody>
</table>

After 5 months of oongrowing, the fish from the different ponds were recorded for live body weight and classified by scale cover type. Only the fish from the MNS group were further oongrown to 22 months of age for intermuscular bones counting.

2.3. Counting of intermuscular bones

A 22 months of age, 10 fish from each of the scale cover types (Mirror, Nude, Scaly) were randomly sampled from the MNS pond. They were individually weighed, then slaughtered and fileted. The right and left filet of each fish were placed in a plastic bag, which was cooked for 10 min in boiling water. Once cooked, each filet was dissected with forceps and scalpel. All bones were extracted, and classified as Y-shaped or regular, and identified to the part (ventral or dorsal) of the filet they were extracted from.

2.4. Data analysis

Differential survival between mirror and nude carps was tested within each pond with a chi-square test comparing the number of survivors from each type, which was supposed to be equal in case of absence of differential survival. This was true both for the pre-growing phase, where the segregation of the N allele was supposed to produce an equal amount of nude and mirror phenotypes, and for the grow-out phase where equal initial numbers of nude and mirror juveniles were seeded in each pond.

Body weight differences were tested within pond with a Student test, using individual body weight as the experimental unit. Global comparison of means between ponds for body weight, biomass or biomass gain were done with a paired Student test or a Wilcoxon signed-rank test, using the pond mean value of a given genotype as the experimental unit. In the paired Student test, the pairs were the mean value of the nude and the mirror genotype from each pond. The Wilcoxon signed-rank test was used to combine biomass gain data from ponds at different stages, where no hypothesis can be done on the distribution of the residual and non-parametric tests are then warranted.

The number of different categories of intermuscular bones between nude, mirror and scaly carps was studied with a one-way ANOVA for each category of bones. Data were analyzed with software R, version 2.14.1.

3. Results

3.1. Nursery performance

Some problems with a broken levee in pond L2 led to contamination by a nearby pond which contained mirror and scaly carps in unknown proportions. Therefore, it was not possible to precisely assess neither the number of surviving mirror carps, nor their mean body weight. Consequently, the data from pond L2 could not be used in the analysis.

Growth, survival and biomass data from the remaining two ponds are given in Table 1.

Average survival was 26.9% on average in pond P, and 33.5% on average in pond L1. Survival of mirror carps was higher than that of nude carps in pond P (P < 0.001) but not in pond L1 (P = 0.07). Within pond, mirror carps were heavier than nude carps in pond P (P< 0.05) but again not in pond L1 (P= 0.16). Biomass and biomass per seeded larva were always higher (>27–69%) for mirror carps, however this difference was not significant (paired Student test, P > 0.27), probably due to the lack of adequate replication.

3.2. Grow-out performance

No results were available for pond P1, as it dried out accidentally due to a rupture of the dam 19 days after the initiation of the experiment. The growth and survival results for the other ponds are given in Table 2.

Survival between the mirror and nude genotypes was different, in favor of mirror carps, in pond MNS and rice field R2, while it was similar in pond P2 and rice field R1. Final body weight was similar between the two genotypes within each rearing unit (Student test, P > 0.05) except in rice field R1 where mirror carps were larger (Student test, P < 0.01). At the between-ponds level, mean final body weight was similar for both genotypes (paired Student test, P > 0.7). As there were differences in stocking weight of mirror and nude carps within ponds, although it was not significant between ponds (paired Student test, P > 0.15), we also estimated the amount of biomass gained for each juvenile stocked, for which the absolute value was higher for mirror carp than for nude carp in all rearing structures. However this difference was not significant with the paired Student test (P = 0.12).

Taking together pond means in biomass production by stocked fish (which combines growth and survival), both in the nursery and
the grow-out stage, there were in total data from six ponds available, and in all of them the biomass production per stocked fish from the nude genotype was lower than that from the mirror genotype, on average 57.4% lower (18.8 to 84.4% lower). This difference was significant ($P = 0.03$, Wilcoxon signed-rank test).

### 3.3. Intermuscular bones

Ten carps of each of the genotypes (mirror, nude scaly) were evaluated for number of intermuscular bones (Table 3).

<table>
<thead>
<tr>
<th>Scale cover type</th>
<th>Nude ($n = 10$)</th>
<th>Mirror($n = 10$)</th>
<th>Scaly($n = 10$)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IM bones</td>
<td>100.3 (2.7)</td>
<td>100.3 (4.1)</td>
<td>100.2 (3.6)</td>
<td>0.98</td>
</tr>
<tr>
<td>Ventral IM bones</td>
<td>33.3 (3.0)</td>
<td>33.3 (2.4)</td>
<td>32.6 (2.8)</td>
<td>0.84</td>
</tr>
<tr>
<td>Dorsal IM bones</td>
<td>67.4 (3.4)</td>
<td>67.0 (3.2)</td>
<td>67.6 (3.4)</td>
<td>0.92</td>
</tr>
<tr>
<td>Y-shaped IM bones</td>
<td>41.8 (13.1)</td>
<td>45.8 (6.3)</td>
<td>45.9 (7.1)</td>
<td>0.99</td>
</tr>
<tr>
<td>Straight IM bones</td>
<td>58.7 (13.9)</td>
<td>54.5 (6.9)</td>
<td>54.3 (9.1)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

### 4. Discussion

This study is the first one in Madagascar to evaluate the performances of different genotypes of common carp in field conditions. Working on the field has advantages in terms of applicability of the results generated, which then reflect real farming conditions. It is also expected to improve results uptake by the farmers. Still, field experiments also have drawbacks (contamination between ponds, dam rupture). In our case, this led to the loss of one replicate in the nursery phase, and of another replicate in the grow-out phase, leading to sub-optimal statistical power. Still, we were able to obtain significant results on our working hypotheses. In any case, there is no functional aquaculture experimental station allowing sufficient replication in Madagascar, so field experiments were indeed our only option to answer the questions raised by Malagasy fish farmers.

However, we used the segregation of the N gene in the offspring of a nude *×* mirror cross to generate a “common garden” design, where all fish are from the same parent batches, spawned on the same day, and only differ by the fact that they possess or not an N allele. Equal numbers of nude and mirror offspring were supposed to be present in the initial batches of larvae, following the results reported by Kirpichnikov (1999). In this case, this allows following the differential mortalities from fertilization to the fingerling stage where the nude and mirror phenotypes can be easily recognized. Recent results have however hypothesized that the Kirpichnikov model could be an over-simplification of reality, and while the S gene was clearly identified as a paralog of fgfr1 (Rohner et al., 2009), the N gene seems less clearly defined, with nude *×* mirror crosses (like the ones we used here) producing in some cases a large excess of mirror phenotypes (Casas et al., 2013). Still, this excess of mirror phenotypes was observed only with one specific origin (Hungarian) for the mirror and nude fish used. In our case, both evaluated groups were constituted by the same initial batch of eggs, and one of them gave almost equal numbers of mirror and nude fish in the nursery phase, so we consider likely that in our case, the “classical” Kirpichnikov segregation model for the N gene is effective, as it is in common carp of Asiatic origin (Casas et al., 2013).

In terms of survival, we did not observe a significantly better survival of nude fish in any rearing unit, neither in the nursery phase, nor in the grow-out phase. On the contrary, we observed significantly better survival of the mirror fish in one pond in the nursery phase, and in one pond and one rice field in the grow-out phase. Although we do not have an adequate statistical power to globally test survival across our different ponds, this is an important indication on the relative value of the mirror and nude genotypes in terms of survival. On average, survival was 18% lower in nude compared to mirror in the nursery stage, while it was 40% lower in the grow-out stage. These figures compare well with nude carps exhibiting a survival relative to mirror carps ranging from −12% (favorable conditions) to −60% (unfavorable conditions), as reported by Kirpichnikov in 1945 in Russia (cited in Kirpichnikov, 1999). The grow-out phase was not done in very favorable conditions here, as shown by the rather small weight gain. Typical ponds and rice fields used by Malagasy farmers for fish culture are small, and we stocked quite a high number of fish for ongrowing (1 fingerling/m²) to ensure sufficient statistical power—but this resulted in low growth, especially due to the fact that the farmers were not very prone to using supplemental feed.

In terms of growth, the results were also questionable, but there again the only significant differences recorded within some ponds (one in the nursery phase, one in the grow-out phase) were in the advantage of the mirror genotype. In previous studies, a reduction in growth of 15–25% in nude carps compared to mirror carps was shown (reviewed by Kirpichnikov, 1999). Here, we cannot say that nude carp has a lower growth than mirror carp in general, and in some cases, although these differences were not significant within pond, we could see higher mean body weights in nude carps at the end of the grow-out stage. Still, combining growth and survival, and the nursery and grow-out phases, we could show that in all ponds the biomass production per fish stocked was lower for nude carps than it was for mirror carps, and that this difference was significant. Therefore, we can conclude that the global productivity of nude common carp in Malagasy conditions is inferior to that of mirror carp, and that mirror carps should thus be preferred for carp farming in Madagascar, as is the case in Europe (Hollebeq and Haffray, 1994; Kirpichnikov, 1999).

Intermuscular bones are located in the myosepta of lower teleosts, and are a serious limit to the organoleptic perception and consumption of carp (Cao et al., 2015; Valloard and Arthaud, 2009). Our average number of IM bones per fish (100) was similar to the values recorded by previous studies (Meske, 1968; Moav et al., 1975; Valloard and Arthaud, 2009), and we found no variation between the nude and the mirror genotypes, which were just differing by the presence/absence of the N allele, on the same genetic background. It has been shown that the reduction of number of scales in carps (implied by the nude phenotype) could also increase the number of fin deformities of decrease the number of pharyngeal teeth (Casas et al., 2013), but no effect on intermuscular bones has been shown before. It has been shown before that different strains of carp could have different numbers of IM bones (Cao et al., 2015; Moav et al., 1975). It may then be that the fact that nude carps are thought to have less bones than mirror carps in Madagascar could be linked to the presence of different strains with different proportions of nude and mirror carps—and that the difference between strains would be attributed to the nude phenotype. Testing this would require sampling more fish from more strains, but in any case the present study proves that it is not the N gene *per se* that causes a reduction in the number of intermuscular bones.

The last supposed benefit of nude carps in their ease of preparation due to the absence of scales. This limitation of scales number is
the main characteristic of this genotype, and if this is found advantageous, it must be taken into account. Still, there are strains of mirror carps with very limited numbers of scales (Steffens, 2008), which would have almost the same advantage, without the negative pleiotropic effects of the N gene. In Madagascar, we have shown in another experiment that the number of scales in mirror carps was heritable (M. Vandeputte, unpublished work)—and thus it would be possible to perform phenotypic selection for reduced scale number if this appears to be a trait of high interest.

This field experiment in Madagascar allowed us to conclude that the productivity of the nude genotype of the common carp was inferior to that of the mirror genotype, using a common garden experiment with segregation of the N genotype. We also showed that the supposed benefits of nude carps in terms of reduced number of IM bones were not justified. Therefore, we can recommend the use of mirror rather than nude carps in Malagasy fish farming, with the option to look for mirror phenotypes with reduced scale number to obtain fish that are better appreciated by the consumer.

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