Ecological adaptation of an F1 hybrid cross of carnivorous and herbivorous Cyprinidae fishes

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Abstract:

Background: Whether hybridization plays a positive or negative role in speciation remains a controversial issue to date. Genetic factors have been widely studied, but ecological factors also play an important role. Although studies on the ecological adaptation of hybrids between different niche parents have been widely reported, cases of extreme niche parental hybridization have not been documented, which may show more ecological phenomena in the fields of hybrid speciation and ecological species isolation.

Results: Taking Cyprinidae fish parents (*Schizothorax wangchiachii* and *Percocypris pingi*) with extreme ecological niches (herbivorous and carnivorous) and their F1 hybrids as research objects, fish, shrimp, blood worms and periphytic algae were selected as food correspond to four different ecological niches. Morphologically, most external and skeletal traits in the F1 hybrids were balanced between the parents, but digestive traits were closer to those of herbivorous parents. In terms of diet, the F1 hybrids weakly foraged for parental food resources, but can more effectively forage for intermediate food resources. In foraging abilities, the F1 hybrids showed low foraging enthusiasm and abilities for parent resources, although the former was the more important factor. Interestingly, the F1 hybrids showed high foraging enthusiasm and success rates when they first foraged for fish, but then they vomited fish debris as a result of mechanical difficulty in chewing rather than taste, and the reason was a contradiction between the genetic behaviours and
intermediate morphology. This behaviour was harmful and was persistent in
some individuals, representing a new mechanism in ecological species
isolation. However, the F1 hybrids have also shown evidence of new
ecological niche formation in favour of hybrid speciation by abandoning
foraging parent resources and focusing more on foraging intermediate foods.

**Conclusions:** (1) Low foraging enthusiasm is an important reason for the
fitness decrease of F1 hybrids to parent food. (2) The contradiction between
genetic behavior and intermediate traits is reported for the first time. (3) F1
hybrids may form an intermediate ecological niche between parents proved
experimentally.

Key-words: Hybrid speciation, ecological isolation, new ecological niche,
foraging behavior, hybrid fitness

1. Introduction

What role does hybridization play in speciation? Some researchers
thought that hybridization was an evolutionary dead end [1], because hybrids
were more often observed to be less healthy and ecologically adapted than
either parent species, and tended to be sterile [2-7]. However, others indicated
that hybridization can provide an important source of genetic variation on
which selection might act and that its adaptive role was more widespread
[8-16]. The survival and adaptation of F1 hybrids is the first and most important
step in hybrid speciation and is affected by both genetic and ecological challenges. Genetic challenges refer to the low fitness of hybrids due to the prevalence of genetic incompatibility between the genomes of different populations [4, 7, 17]. Ecological challenges refer to reduced hybrid fitness due to the maladaptive intermediacy of their ecologically relevant genotypes and phenotypes in parental environments [3, 18, 19]. Genetic challenges have been widely reported, but ecological challenges also play an important role [17, 20].

Morphology is often determined by quantitative traits, therefore the morphology of F1 hybrids are general between parents [19, 21]. If no intermediate ecological niche exists between the parents of the F1 hybrids, they often show low fitness for parent resources. For example, in zooplankton-feeding and benthic-feeding sticklebacks [18], and poplar and willow leaf beetles [20], intermediate niches are often lacking between the monotrophic parents because identifying transitional species between benthic species and zooplankton and between different plants is difficult. However, when intermediate ecological niches exist between hybrid parents, the results may be different, and the hybrids may develop new ecological niches [15, 22, 23].

Of course, species foraging type is determined not only by traits but also by foraging behaviour, with a significant correlation between the two [24]. Foraging traits are often quantitative, and are therefore frequently additive
between parents in F1 hybrids. However, many unique behaviours develop among species, and these unique parental genetic behaviours of F1 hybrids may be codominant [25] or dominant [26] rather than additive. Therefore, unique parental foraging behaviour is usually dominant rather than additive in F1 hybrids, and the traits of the parents are often additive in the F1 hybrids, which is obviously contradictory.

Due to the primitive evolutionary status of fish and in vitro fertilization, more cases of hybridization are observed in fish than in higher vertebrates [12]. Moreover, similar to plants, fish have many polyploids, especially Cyprinidae, and this trait is often associated with the formation of allopolyploids by ancient distant hybridization that can instantly create new species that are reproductively isolated from their parents [27-31] indicating that hybridization between parents with two different ecological niches will be more likely, and genetically stable new species may form in Cyprinidae.

The species used in this study were cold-water Cyprinidae fishes from the upper Yangtze River basin in the south-eastern Tibetan Plateau. *Schizothorax wangchiachii* (SW) has a sharp horny front jaw and mainly scrapes and eats periphytic algae from rocks. *Percocypris pingi* (PP) is a typical carnivorous fish with a sub-superior mouth. Morphologically, the *Schizothorax* genus and *Percocypris* genus were once thought to belong to two different subfamilies [32]. However, molecularly, they were shown to be sister genera in a recent study [28, 33]. They are homogeneously distributed and have similar breeding
periods, and we have successfully bred healthy F1 hybrids (P. pingi ♀×S. wangchiachii ♂, PS) through artificial hybridization, but backcrossing has failed [34]. Compared with PP, SW has a unique foraging behaviour of scraping and eating algae from rocks (Supplementary Movie 1). Similarly, compared with SW, PP has a unique foraging behaviour of hunting and ambushing prey (Supplementary Movie 2). Although we did not find their natural hybrids, such hybridization between parents with two extreme ecological niches is more likely to yield some valuable and distinct ecological phenomena than parents with small ecological niche differences in the fields of hybrid speciation and ecological species isolation.

In this study, carnivorous fish, herbivorous fish and their F1 hybrids were used to explore the ecological adaptability of the F1 hybrids through comparative behavioural and morphological studies to provide new theoretical results for related studies on the isolation of ecological species and speciation by hybridization.

2. Materials and methods

2.1 Experimental fish acquisition

In March 2017 and 2019, a hybridization experiment and parental reproduction were carried; for details on the methods, refer to the research by [34]. Age-two fishes (PP (122.03±1.78 mm, 25.2±1.05 g), SW (106.78±1.41 mm, 18.43±0.74 g) and PS (125.84±2.71 mm, 29.22±1.85 g)) were used to
quantify both external and skeletal characteristics, and age-one fishes (PP (9.08±0.34 mm, 12.07±0.90 g), SW (9.23±0.14 mm, 13.03±1.50 g) and PS (9.17±0.48 mm, 14.02±3.76 g)) were used to quantify digestive characteristics, foraging and behavioural features.

2.2 Morphology

The external morphology of age-two SW (n=30), PP (n=30) and PS (n=30) was studied, and the examination standards were referenced from [35]. Then, we selected 10 fish individuals for quantification of skeletal morphology. Their opercular bone, pharyngeal bone, dentary bone and skull were obtained by boiling, and the examination standards are described in Supplementary Fig. 1. Next, the digestive characteristics of age-one SW (n=6), PP (n=6) and PS (n=6) were studied. This study quantified the anatomy and histology (Hematoxylin-eosin staining) of the digestive organs, and the examination standards are described in Supplementary Fig. 2. Finally, 19 external morphological indicators, 19 skeletal morphological indicators and 23 digestive indicators were quantified in this study, as shown in Supplementary Tables 1-2. To visually show the comprehensive morphological differences of the three fishes, we conducted principal component analysis (PCA) of the Z-scores of three categories of indicators in SPSS 21.0.

The body shapes were photographed using an SLR camera (Canon EOS 100D, Japan). The details of the heads fixed by Bouin's fixative and bones were photographed (Figs. 1 and 3) by a stereomicroscope (Nikon SMZ25).
Slices of the digestive organs were photographed (Fig. 2) under a microscope (Nikon ECLIPSE 80i). Age-two PP, SW and PS were scanned (Fig. 3) using a MicroCT Skyscan 1176 (Bruker, Belgium) to obtain the holistic bone structure; specific methods are described in [36], and it were slightly modified in this study.

2.3 Comparison of foraging habit

We fed PP, SW and PS with small fish (*Sinibrama taeniatus*, 0.0507±0.0043, a cyprinid fish that can breed in our lab year round (Fig. 4a)), small shrimp (*Neocaridina denticulate*, 0.1093±0.0227, which is widely distributed in China's rivers (Fig. 4c)), Tubificidae worms (an aquatic mollusc (Fig. 4b)) and periphytic algae (*Spirogyra*, a filamentous algae (Fig. 4d)), which correspond to foods in different ecological niches. Specific experimental methods can be found in Supplementary method 1. We compared each fish species' foraging level (FL) using the following formula:

\[
FL = \frac{M2}{M1 - M2}
\]

where \( M1 \) represents body weight, and \( M2 \) represents chyme weight.

2.4 Hybrid vs *P. pingi* in foraging fish

We compared the foraging capacity of PP (n=15) and PS (n=18) for small fishes (*S. taeniatus*) (Fig. 5a). Specific experimental methods are described in Supplementary method 2. We observed experimental fishes by video and quickly replayed the video and counted the following indicators: first attack time (FAT), first success time (FST), the success rate of the first attack (SRFA),
first attack time after the first successful capture (FAT2), attack frequency (AF), the success rate of the total attacks (SRTA), and the rate of vomiting fish (RVF).

Details are as follows:

FAT: The time when an experimental fish first attacked the small fishes. To exclude the influence of irritability, only the experimental fishes that launched the first attack within 5 min were included in all statistical comparisons.

FST: The time when an experimental fish first successfully caught a small fish. If it did not succeed within 30 min, a value of 30 min was used as its first success time.

SRFA: The success rate when an experimental fish first attacked the small fishes.

FAT2: The time when an experimental fish first attacked after the first successful capture.

AF: The average number of attacks per minute of an experimental fish; this value was calculated using the following formula:

\[ AF = \frac{N}{T} \]

SRTA: The success rate of the total attacks; this value was calculated using the following formula:

\[ SRTA = \frac{N'}{N} \]

RVF: The rate of vomiting fish; some individuals catch fish and then vomit them out; this value was calculated using the following formula:

\[ RVF = \frac{N''}{N'} \]
where $N$ represents the total number of attacks; $T$ represents the time at the end of the experiment; $N'$ represents the total catch before the end of the experiment; and $N''$ represents the number of fish vomited.

2.5 Hybrid vs S. wangchiachii in foraging periphytic algae

We compared the abilities of SW (n=16) and PS (n=20) to forage periphytic algae (Fig. 5b). Specific experimental methods are described in Supplementary method 3. We quickly replayed the video and evaluated the following indicators: The FAT, AF, and foraging efficiency (FE). Details are as follows:

FAT: The time when an experimental fish first scraped periphytic algae from the rocks.

AF: The average number of scrapings per hour of experimental fish; this value was calculated using the following formula:

$$TAF = (N_2 + N_5 + N_8)/3$$

FE: The average weight of a single scrape of periphytic algae per unit weight of experimental fish; this value was calculated using the following formula:

$$EF = M_2/(TAF \times 8 \times (M_1 - M_2))$$

where $N_2, N_5,$ and $N_8$ represent the number of attacks in the second, fifth and eighth hours, respectively, $M_1$ represents the body weight of the experimental fish; and $M_2$ represents the chyme weight of the experimental fish.

2.6 Whether the behaviour of hybrid fish vomiting fish is persistent
In the previous experiments, we observed that PS had obvious behaviour of vomiting fish (Fig. 4e and Supplementary Movie 3). This behaviour is very interesting and important but is this behaviour persistent? We set up a feeding experiment using small fish (*Carassius auratus*, 0.0748±0.0023 g (Fig. 6a)) for nine days, and PS still had obvious vomiting behaviour after catching the small *C. auratus* fishes (Fig. 6c). For nine days, we fed not only fish, but we also fed the blood worms (0.0171±0.0006, Chironomidae larvae, a soft-bodied aquatic insect that is easier to count and preserve than Tubificidae worms (Fig. 6b)), to simulate a palatable food shortage in the natural environment but not a complete absence. Specific experimental methods are described in Supplementary method 4. We counted the daily catch, intake, and vomiting of each PS for small fish.

2.7 Mechanism explaining why hybrid fish vomited fish

Two mechanisms may explain why PS vomited small fish: the small fish tasted bad or they were difficult to chew. To explore this mechanism, we selected approximately 50 g of *C. carp* (Fig. 7a) and cut the back muscle into small pieces (Fig. 7b) without bone, instead of using small fish. We took PS that had the obvious behaviour of vomiting small fish in the last experiment as the experimental fishes (n=7). Other than the small fishes that were replaced with small pieces of *C. carp* muscle, the other feeding and statistical schemes were the same as those in Section 2.6. However, the experiment lasted only three days. We counted the average number of daily foraging (ANDF) and the
vomiting rate (VR) of the 7 experimental fishes used in Section 2.6 and this experiment, which was equivalent to the former serving as a control group for the latter, by the following formulas:

\[
\text{ANDF} = \frac{N}{T} \\
\text{VR} = \frac{N'}{N}
\]

where \(N\) represents the total number of preys captured by PS during the experiment. \(T\) represents the number of days of the experiment, and \(N'\) represents the total number of these fishes that vomited.

Then we compared the pharyngeal teeth details of PP, SW and PS, and quantified the foraging-related traits (Supplementary Table 9, 20 measured traits and 17 standardized traits) of all fishes (\(n=32\)) in Section 2.6 to explore whether a correlation exists between these traits, and these indicators included the TNC (total number of captures), TNI (total number of ingestions), NVF (total number of vomiting fish) and RVF by Spearman’s correlation in SPSS 21.0.

2.8 New ecological niche formation of hybrid fish

In previous experiments, we found that PS was unable to efficiently forage for both small fish and periphytic algae. Therefore, we questioned whether PS focused more on foraging intermediate foods, thereby forming a new ecological niche different from its parents. We tested effective attacks on blood worms per species in a mixed breeding experiment under conditions of only blood worms or mixed foods. Specific experimental methods are described in
Supplementary method 5. To eliminate differences in each parallel group, we
standardized the foraging weight of each fish in each tank and calculated the
foraging proportion (FP) by the following formula:

\[ FP = \frac{N'}{N} \]

where \( N \) represents the total number of effective attacks on blood worms by
three fishes in a tank, and \( N' \) represents the total number of effective attacks
on blood worms by a fish species in a tank.

3. RESULTS

3.1 Morphology

Regarding the external and skeletal morphology, most PS traits were
between PP and SW. However, for digestive morphology, PS was closer to SW
(Figs. 1-3 and Supplementary Table 2). Direct observation, Tukey test or PCA
all supported the above data. Specific morphological descriptions are provided
in Supplementary result 1.

3.2 Comparison of foraging habits

In Fig. 4, for small fish, the FL of PP (0.0521±0.0170) was significantly
higher (\( P<0.05 \)) than those of SW (0.0012±0.0014) and PS (0.0038±0.0070).
The latter two ingested very few small fishes, and no significant difference
(\( P\geq0.05 \)) was found between them. For shrimp, the FL of PP (0.0563±0.0197)
was significantly higher (\( P<0.05 \)) than those of SW (0.0126±0.0099) and PS
(0.0239±0.0225). PS and SW ingested more shrimp than fish, and the former
ingested more shrimp than the latter, but no significant difference was noted
between them \((P \geq 0.05)\). For Tubificidae worms, the FLs of PP 
\((0.0297 \pm 0.0144)\), SW \((0.0290 \pm 0.0196)\) and PS \((0.0327 \pm 0.0213)\) were similar, 
with no significant difference between them \((P \geq 0.05)\). For periphytic algae, the 
FL of SW \((0.0110 \pm 0.0046)\) was significantly \((P < 0.05)\) higher than those of PP 
\((0.0000 \pm 0.0000)\) and PS \((0.0035 \pm 0.0051)\). PP did not ingest periphytic algae, 
and some PS individuals may have ingested a small amount of periphytic 
algae, but no significant difference was observed between them \((P \geq 0.05)\). SW 
had a low intake of periphytic algae, possibly because this study used only 
Spirogyra instead of more palatable diatoms.

Interestingly, we found a large amount of small fish debris in the PS 
aquarium tank (Fig. 4e), while less debris was noted in the tanks with SW and 
PP, suggesting that one of the reasons why fish intake by PS intake was low 
was vomiting of fish.

3.3 Hybrid vs parents in foraging fish or periphytic algae

In the PS vs SW experiment, the FAT of PS was extremely significantly 
higher \((P < 0.01)\) than that of SW (Fig. 5b), the AF was significantly lower 
\((P = 0.02)\) than that of SW (Fig. 5c), and the FE was significantly lower \((P = 0.037)\) 
than that of SW (Fig. 5e). In summary, PS showed low interest in foraging for 
periphytic algae and had low foraging efficiency.

In the PS vs PP experiment, the SRFA \((P = 0.219)\) and the SRTA \((P = 0.167)\) 
of PS were not significantly different from those of PP; the RVF of PS was
extremely significantly higher (P<0.01) than that of PP (Fig. 5e); the FAT (P=0.459) and the FST (P=0.161) of PS were not significantly different from those of PP; the FAT2 was extremely significantly higher (P<0.01) than that of PP (Fig. 5f); and the AF of PS was extremely significantly lower (P<0.01) than that of PP (Fig. 5g). In summary, PS showed greater interest in first foraging for fish but had a high RVF, which caused it to be negative in later predation.

3.4 Whether the behaviour of hybrid fish vomiting fish is persistent

As shown in Fig. 6, at the beginning of the experiment, most PS had the behaviours of catching, vomiting and ingesting small fish. However, as the experiment proceeded, the number of PS with these behaviours decreased, and only a few fish retained these persistent behaviours by the end of the experiment (Figs. 6d, 6e, 6f and 6j); thus, this pattern was the main reason for the decline in the average number of daily captures, vomiting and ingestion (Figs. 6g, 6h and 6i). In summary, the behaviour of catching, vomiting and ingesting small fish by most PS were not persistent.

3.5 Mechanism of hybrid fish vomiting fish

No significant difference (P=0.702) was found between the ANDF of fish meat and small fish in the individuals exhibiting persistent capture behaviours. However, the VR of fish meat was significantly lower (P<0.01) than that of small fish, suggesting that the vomiting behaviour was not caused by bad taste but by chewing difficulty, which may be caused by pharyngeal tooth structure. Therefore, the details of the pharyngeal teeth were compared, and we found
that the pharyngeal bone of PP was long and narrow, with widely spaced well-developed conical hooked pharyngeal teeth, and the space was larger between the two pharyngeal bones in the closed mouth. These features are useful for piercing and hooking prey. In contrast, the pharyngeal bone of SW was short and thick, with closely spaced grinding pharyngeal teeth, which were curved and flat at the top, forming a grinding surface, and the space was smaller between the two pharyngeal bones in the closed mouth. These features are useful for grinding periphytic algae. The morphology of the pharyngeal bone in PS was balanced between that of the parents, and it had hooked grinding pharyngeal teeth, which were also intermediate between the parents.

Specific correlation analysis descriptions can be found in Supplementary result 2. The results of the correlation analysis can be summarized as follows: (1) as the number of fishes caught by PS increased, more were ingested and vomited. (2) The larger the PS was, the more fishes it caught. (3) The behaviour of vomiting fish by PS was not correlated with the size and shape of its characteristics.

3.6 New ecological niche formation of hybrid fish

In the first 3 days of feeding using only blood worms, PS and SW showed no significant difference (P≥0.05) in the FP of blood worms, but they significantly differed (P<0.05) from PP (except that PS showed no significant difference (P≥0.05) compared to SW and PP on the second day, Fig. 8d). After
3 days of feeding with three kinds of food, the FP of PP with 3 days of blood worm feeding decreased significantly (P=0.036), and that of SW decreased nonsignificantly (P=0.168); however, that of PS increased significantly (P=0.01, Fig. 8e). Moreover, the FP was significantly (P<0.05) different among PS and both parents in the first two days of feeding on the three kinds of food, but PS and SW showed no significant difference (P≥0.05) on the third day (Fig. 8d).

4. Discussion

Hybrid speciation needs to break through multiple isolation barriers, which are generally divided into prezygotic and postzygotic barriers [17]. Random external fertilization by medium makes it easier for plants and fishes to cross, which is beneficial to break through the pre-zygotic barrier [12]. Polyploid speciation also facilitates post-zygotic breakthroughs [10]. However, all above breakthroughs are genetic, for hybrid speciation, ecological challenges must be faced and foraging performance is the first barrier.

The feeding habits of a species are closely related to their foraging traits [37]. PP and SW have opposite feeding habits and foraging traits. With the exception of a few epistatic traits, most foraging traits of PS are somewhere between the traits of their parents (Figs. 1-3). In general, hybrids will produce intermediate foraging performance for parent resources [18-20, 23, 38]. However, intermediate kinematics have also been shown produce more inferior foraging performance than intermediate morphology [39]. In this study, the intermediate foraging morphology of PS did not result in intermediate
foraging performance for parental resources, and PS could hardly forage for parental resources (Figs. 4f and 5). The above phenomenon was caused not only by the decline in PS foraging ability but, more importantly, by the decrease in foraging activity (Fig. 5). The diet of a species depends not only on heredity and environment, but also experience [40-45]. PS showed less interest in foraging for periphytic algae from the beginning of the experiment, which may be genetically negative. Interestingly, however, PS showed interest in foraging small fishes at the beginning of the experiment, while after the first successful capture, PS had difficulty ingesting the fish, which led to subsequent negative predation (Fig. 5). This result may be empirically negative.

The behaviour of PS vomiting fish is one of the highlights of this study. Some vomiting events are caused by an individual's ingestion of stimulating foods, which may be due not only to unpalatably or toxicity [46] but also to mechanical issues, such as dolphins ingesting hard squid beaks, resulting in vomiting [47]. Our experiments proved that the vomiting behaviour of PS was caused not by bad taste but rather by chewing difficulty, which may be caused by mechanical difficulties encountered due to the bones of small fish (Fig. 7).

In the correlation analysis, we found no correlation between the structure of any trait in PS and this behaviour (Supplementary Table S9). However, the structure of pharyngeal teeth of PS was balanced between that of its parents, but its puncture ability was not as good as that of PP; thus, PS may not reach the threshold of normal chewing (Fig. 7). Of course, most PS individuals
abandoned foraging fish, but some showed persistent vomiting behaviour (Fig. 6). Clearly, the behaviour itself is harmful as it requires energy that PS could expend on foraging, and PS consequently does not receive the intended source of energy. In short, PS must engage in hard work with no gain. Thus, we hypothesized that these contradictory foraging behaviours may dilute the energy of PS intended for foraging intermediate ecological niche prey, and a supplementary experiment was therefore carried out (Supplementary method 6 and Supplementary result 3). However, we did not find a correlation between the amount of vomited fish and the total food intake of PS (Supplementary Table 11), which contradicts the above hypothesis. Interestingly, both SW and SP showed reduced predation of shrimps and worms in the food resource environment with fishes, suggesting that small fish were interfering with their predation. However, SP showed greater reductions than SW, which may be due to the negativity brought by vomiting fish (Fig. 5 and Supplementary Fig. 3).

The periphytic algae will not affect the foraging of PS for intermediate ecological niche prey because PS is less interested in foraging on such algae and has less interference (Fig. 5 and Supplementary Fig. 3). In summary, the behaviour of PS vomiting fish is a typical paradoxical phenomenon between intermediate morphology and genetic behaviour, which led directly or indirectly to the ecological disadvantage of PS.

PS almost abandoned foraging on parental resources and could only choose intermediate ecological niche prey, which may indicate that PS forms a
middle ecological niche. We experimentally verified the above hypothesis that the foraging preference of PP and SW for intermediate ecological niche prey decreases after the addition of suitable parental food resources, whereas that of PS increases (Fig. 8). If no transitional food resources exist between parents, hybrids will be eliminated [3, 20]. Instead, hybrids may form a new ecological niche [15, 23]. Through a mixed breeding experiment, this study showed that the increase in the FP of PS to intermediate ecological niche prey was not due to the adaptation of new traits [23] and was more likely due to the decrease in the FP of the parents to such prey. However, this is a type of passive ecological niche formation, indicating that more competitors are likely to participate.

When there are transitional ecological niches exist between different ecological niche parents, the hybridization between them may be a double-edged sword for hybrid speciation, implying that the fate of hybrids is complicated in this process. The experimental period of this study was short, but the formation of a stable niche is long. Therefore, large-scale and long-term bionic breeding experiments and stable isotope methods [19] should be carried out in subsequent studies to verify whether hybrids form new niches or are eliminated.
Ethics approval and consent to participate

The authors claim that none of the material in the paper has been published or is under consideration for publication elsewhere. The submission is original, and all authors are aware of the submission and agree to its publication in Frontiers in Zoology. We declare that there is no conflict of interests regarding the publication of this paper.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no competing interests.

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Authors' contributions

H.R. Gu and Z.J. Wang conceived the ideas and designed the methodology; Y. He took the microCT images; S.H. Deng, X.H. He, Y. Wu, K.Y. Xing and X. Gao contributed to the breeding and feeding of the experimental
fishes used in this experiment; H.R. Gu completed all the experiments and
data processing and analysis in this study; X.F. He provided constructive
guidance to H.R. Gu in morphology; H.R. Gu and Z.J. Wang led the writing of
the manuscript. All authors contributed critically to the drafts and provided final
approval for publication.
References

1. Mayr E: *Animal species and evolution*. Cambridge: Belknap Press; 1963.
2. Orr HA, Turelli M: The evolution of postzygotic isolation: Accumulating Dobzhansky-Muller incompatibilities. *Evolution* 2001, 55:1085-1094.
3. Schluter D: *Adaptive Radiation In Sticklebacks - Trade-Offs In Feeding Performance And Growth*. *Ecology* 1995, 76:82-90.
4. Powell DL, Garcia-Olazabal M, Keegan M, Reilly P, Du K, Diaz-Loyo AP, Banerjee S, Blakkad D, Reich D, Andolfatto P, et al: Natural hybridization reveals incompatible alleles that cause melanoma in swordtail fish. *Science* 2020, 368:731-+.
5. Schluter D: Evidence for Ecological Speciation and Its Alternative. *Science* 2009, 323:737-741.
6. Satokangas I, Martin SH, Helantera H, Saramaki J, Kulmuni J: Multi-locus interactions and the build-up of reproductive isolation. *Philosophical Transactions Of the Royal Society B-Biological Sciences* 2020, 375.
7. Brideau NJ, Flores HA, Wang J, Maheshwari S, Wang X, Barbash DA: Two Dobzhansky-Muller genes interact to cause hybrid lethality in Drosophila. *Science* 2006, 314:1292-1295.
8. Dowling TE, Secor CL: The role of hybridization and introgression in the diversification of animals. *Annual Review Of Ecology And Systematics* 1997, 28:593-619.
9. Rius M, Darling JA: How important is intraspecific genetic admixture to the success of colonising populations? *Trends In Ecology & Evolution* 2014, 29:233-242.
10. Arnold ML: *Natural hybridization and evolution*. New York: Oxford University Press; 1997.
11. Pfennig KS: *ECOLOGY How to survive in a human-dominated world*. *Science* 2019, 364:433-434.
12. Montanari SR, Hobbs JPA, Pratchett MS, van Herwerden L: The importance of ecological and behavioural data in studies of hybridisation among marine fishes. *Reviews In Fish Biology And Fisheries* 2016, 26:181-198.
13. St John ME, Holzman R, Martin CH: Rapid adaptive evolution of scale-eating kinematics to a novel ecological niche. *Journal Of Experimental Biology* 2020, 223.
14. Selz OM, Thommen R, Maan ME, Seehausen O: Behavioural isolation may facilitate homoploid hybrid speciation in cichlid fish. *Journal Of Evolutionary Biology* 2014, 27:275-289.
15. Masello JF, Quillfeldt P, Sandoval-Castellanos E, Alderman R, Calderon L, Cherel Y, Cole TL, Cuthbert RJ, Marin M, Massaro M, et al: Additive Traits Lead to Feeding Advantage and Reproductive Isolation, Promoting Homoploid Hybrid Speciation. *Molecular Biology And Evolution* 2019, 36:1671-1685.
16. Pfennig KS: Facultative mate choice drives adaptive hybridization. *Science* 2007, 318:965-967.
17. Coyne JA, Orr HA: *Speciation*. Sunderland, MA: Sinauer Associates; 2004.
18. Hatfield T, Schluter D: Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. *Evolution* 1999, 53:866-873.
19. Arnegard ME, McGee MD, Matthews B, Marchinko KB, Conte GL, Kabir S, Bedford N, Bergek S, Chan YF, Jones FC, et al: Genetics of ecological divergence during speciation.

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Nature 2014, 511:307–+

20. Egan SP, Funk DJ: Ecologically dependent postmating isolation between sympatric host forms of Neochlamisus bebbianae leaf beetles. Proceedings Of the National Academy Of Sciences Of the United States Of America 2009, 106:19426-19431.

21. Douglas JF: Evolution. Third Edition. Sunderland, Massachusetts: Sinauer Associates; 2013.

22. Seehausen O: Hybridization and adaptive radiation. Trends In Ecology & Evolution 2004, 19:198-207.

23. Selz OM, Seehausen O: Interspecific hybridization can generate functional novelty in cichlid fish. Proceedings Of the Royal Society B-Biological Sciences 2019, 286.

24. Lopez-Fernandez H, Arbour J, Willis S, Watkins C, Honeycutt RL, Winemiller KO: Morphology and Efficiency of a Specialized Foraging Behavior, Sediment Sifting, in Neotropical Cichlid Fishes. Plos One 2014, 9.

25. York RA, Patil C, Abdilleh K, Johnson ZV, Conte MA, Genner MJ, McGrath PT, Fraser HB, Fernald RD, Streelman JT: Behavior-dependent cis regulation reveals genes and pathways associated with bower building in cichlid fishes. Proceedings Of the National Academy Of Sciences Of the United States Of America 2018, 115:E11081-E11090.

26. Wheatcroft D, Qvarnstrom A: Genetic divergence of early song discrimination between two young songbird species. Nature Ecology & Evolution 2017, 1.

27. Song C, Liu SJ, Xiao J, He WG, Zhou Y, Qin Q, Zhang C, Liu Y: Polyploid organisms. Science China-Life Sciences 2012, 55:301-311.

28. Yang L, Sado T, Hirt MV, Pasco-Viel E, Arunachalam M, Li JB, Wang XZ, Freyhof J, Saitoh K, Simons AM, et al: Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). Molecular Phylogenetics And Evolution 2015, 85:97-116.

29. Wang XZ, Gan XN, Li JB, Chen YY, He SP: Cyprininae phylogeny revealed independent origins of the Tibetan Plateau endemic polyploid cyprinids and their diversifications related to the Neogene uplift of the plateau. Science China-Life Sciences 2016, 59:1149-1165.

30. Oellermann LK, Skelton PH: Hexaploidy In Yellowfish Species (Barbus, Pisces, Cyprinidae) From Southern Africa. Journal Of Fish Biology 1990, 37:105-115.

31. Xu P, Xu J, Liu GJ, Chen L, Zhou ZX, Peng WZ, Jiang YL, Zhao ZX, Jia ZY, Sun YH, et al: The allotetraploid origin and asymmetrical genome evolution of the common carp Cyprinus carpio. Nature Communications 2019, 10.

32. Yue PQ: FAUNA SINICA, Osteichthyes Cypriniformes III. Beijing, China: Science Press; 2000.

33. Wang M, Yang JX, Chen XY: Molecular Phylogeny and Biogeography of Percocypris (Cyprinidae, Teleostei). Plos One 2013, 8.

34. Gu HR, Wan YF, Yang Y, Ao Q, Cheng WL, Deng SH, Pu DY, He XF, Jin L, Wang ZJ: Genetic and morphology analysis among the pentaploid F-1 hybrid fishes (Schizothorax wangchiachii female x Percocypris pingi male) and their parents. Animal 2019, 13:2755-2764.

35. Zou SP, Fang YL, Zhou RQ: Measurement of characters. Inspection of germplasm for cultured fishes, part 3, vol. GB/T 18654.3–2008. China: Ministry of agriculture of the People's Republic of China; 2008.

36. He Y, Chen XY, Xiao TQ, Yang JX: Three-dimensional morphology of the Sinocyclocheilus hyalinus (Cypriniformes: Cyprinidae) horn based on synchrotron
X-ray microtomography. Zoological Research 2013, 34:E128–134.

37. Manning CG, Foster SJ, Vincent ACJ: A review of the diets and feeding behaviours of a family of biologically diverse marine fishes (Family Syngnathidae). Reviews In Fish Biology And Fisheries 2019, 29:197-221.

38. Kirkpatrick M: Reinforcement during ecological speciation. Proceedings Of the Royal Society B-Biological Sciences 2001, 268:1259-1263.

39. Mcgee MD, Reustle JW, Oufiero CE, Wainwright PC: Intermediate Kinematics Produce Inferior Feeding Performance in a Classic Case of Natural Hybridization. American Naturalist 2015, 186:807-814.

40. Jaenike J: Genetic And Environmental Determinants Of Food Preference In Drosophila-Tripunctata. Evolution 1985, 39:362-369.

41. Bolivar VJ, Flaherty L: Genetic control of novel food preference in mice. Mammalian Genome 2004, 15:193-198.

42. Utsumi S, Ando Y, Ohgushi T: Evolution of feeding preference in a leaf beetle: the importance of phenotypic plasticity of a host plant. Ecology Letters 2009, 12:920-929.

43. Sotka EE: Genetic control of feeding preference in the herbivorous amphipod Ampithoe longimana. Marine Ecology Progress Series 2003, 256:305-310.

44. Finestone E, Bonnie KE, Hopper LM, Vreeman VM, Lonsdorf EV, Ross SR: The interplay between individual, social, and environmental influences on chimpanzee food choices. Behavioural Processes 2014, 105:71-78.

45. Turrovincent I, Launay F, Mills AD, Picard M, Faure JM: Experiential And Genetic Influences on Learnt Food Aversions In Japanese-Quail Selected for High Or Low-Levels Of Fearfulness. Behavioural Processes 1995, 34:23-41.

46. Johnson EC, Hill E, Cooper MA: Vomiting in wild bonnet macaques. International Journal Of Primatology 2007, 28:245-256.

47. AU Silva-Jr. JMP, Lizete Jardim; Sazima, Ivan: Vomiting behavior of the spinner dolphin (Stenella longirostris) and squid meals. Aquatic Mammals 2004, 30:271-274.
Figure legends

Figure 1. External characters comparison. (a) The full view of PP. (b-d) The head characters of PP. The full view of SW. (f-h) The head characters of SW. (i) The full view of PS. (j-i) The head characters of SP. (m) The PCA of external characters. The white scale is 1 mm; the black scale is 10 mm. We have used some figures in previous articles (Gu et al. 2019), including (a), (c), (d), (e), (g), (h), (i), (k) and (l).

Figure 2. Digestive characters comparison. (a) The anatomic observation of midgut mucosal folds of PP. (b) The histological observation of liver of PP. (c) The histological observation of foregut of PP. (d) The histological observation of midgut of PP. (e) The histological observation of Hindgut of PP. (f-j) The same description of SW as PP. (k-o) The same description of SP as PP. (p) The PCA of digestive characters. The scale in (a), (f) and (k) is 0.5 mm, in (b), (g) and (l) is 10 μm, in (c), (d), (e), (h), (i), (j), (m), (n) and (o) is 25 μm.

Figure 3. Osteal characters comparison. (a-c) The MicroCT image of head characters of PP. (d) The pharyngeal bone of PP. (e-g) The MicroCT image of head characters of SW. (h) The pharyngeal bone of SW. (i-k) The MicroCT image of side head of PS. (l) The pharyngeal bone of SP. (m) The PCA of osteal characters. The scale of MicroCT images is 6 mm, and the scale of pharyngeal bones is 1 mm.

Figure 4. Comparison of foraging habit. (a) Small fish (S. taeniatus). (b) Tubificidae worms. (c) Small shrimp (N. denticulate). (d) Periphytic algae
(Spirogyra). (e) Small fish debris. (f) The FL of different foods among PP, SW and PS. The scale of all figures is 1 mm. The different superscripts (a, b) above the boxes differ significantly at P < 0.05 based on Tukey test. The boxes give the first and third quartiles, the thick lines give the medians and whiskers indicate means ± SD.

**Figure 5.** Hybrid vs parents in foraging little fish or periphytic algae. (a) Small fish (*S. taeniatus*). (b) Rock with periphytic algae (Spirogyra). (c) PS vs SW in the FAT. (d) PS vs SW in the AF. (e) PS vs SW in the FE. (f) PS vs PP in the SRFA, SRTA and RVF. (g) PS vs PP in the FAT, FST and FAT2. (h) PS vs PP in the AF. The scale is 1 mm. The numbers above the columns give the P-value based on Tukey test, the height give the mean, the thick lines give the medians and whiskers indicate mean ± SE.

**Figure 6.** The changes of the related indicators of foraging fish in hybrid fish with time. (a) Small fish (*C. auratus*). (b) Blood worm (Chironomidae larvae). (c) Little fish debris. (d) The trends of vomiting of every SP with time. (e) The trends of ingestion of every SP with time. (f) The trends of captures of every SP with time. (g) The mean trend of captures of SP with time. (h) The mean trend of ingestion of SP with time. (i) The mean trend of vomiting of SP with time. (j) The trends in the number of SP involved in capture, ingestion and vomiting. In (d), (e) and (f), each line represents an individual. The scale of all figures is 1 mm. The different superscripts (a, b) above the lines differ significantly at P < 0.05 based on Tukey test, and whiskers indicate mean ±
Figure 7. Mechanism of hybrid fish vomiting small fish. (a) Small fish (*C. auratus*). (b) A small piece muscle in the back of *C. auratus*. (c) The ANDF for small fish or meat by these SP with a persistent vomiting-fish behavior. (d) Compare the VR of SP between foraging small fish and meat. (e) The MicroCT image of pharyngeal bones of PP. (f-g) The detail image of pharyngeal bones of PP. (h) The MicroCT image of pharyngeal bones of SW. (i-j) The detail image of reverse pharyngeal bones of SW. (k) The MicroCT image of pharyngeal bones of SP. (l-m) The detail image of pharyngeal bones of SP. (m) The scale in (a) is 1mm, in (b), the meat is 1mm and the fish is 10mm, in (e), (h) and (k) is 2mm, in (f), (g), (i), (j), (l) and (m) is 0.5mm. The numbers above these columns give the P-value based on Tukey test, the height give the mean, the thick lines give the medians, and whiskers indicate mean ± SE.

Figure 8. New ecological niche formation of hybrid fish. (a) Small fish (*C. auratus*). (b) Blood worm (*Chironomidae* larvae). (c) Rock with periphytic algae (*Spirogyra*). (d) The trends of daily FP of PP, SW and SP in an environment with only worms or fishes, worms and periphytic algae. (e) Comparison of total FP of PP, SW and PS between an environment with only worms or with fishes, worms and periphytic algae. The scale of all figures is 1 mm. The different superscripts (a, b) above the boxes or lines differ significantly at P < 0.05 based on Tukey test. The numbers above the columns give the P-value based on Tukey test. The heights of boxes give the mean, the thick lines give the
medians and whiskers indicate mean ± SE.
Figures

Fig. 1
Fig. 4
Fig. 8