

# **Interspecific hybridization can generate functional novelty in cichlid fish**

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## **Abstract**

The role of interspecific hybridization in evolution is still being debated. Interspecific hybridization has on the one hand been suggested to facilitate the evolution of ecological novelty and hence the invasion of new niches and adaptive radiation when ecological opportunity is present beyond the parental species niches. On the other hand, hybrids between two ecologically divergent species may perform less well than parental species in their respective niches because hybrids would be intermediate in performance in both niches. The evolutionary consequences of hybridization may hence be context-dependent, depending on whether ecological opportunities, beyond those of the parental species, do or do not exist. Surprisingly, these complementary predictions may never have been tested in the same experiment in animals. To do so, we investigate if hybrids between ecologically distinct cichlid species perform less well than the parental species when feeding on food either parents are adapted to, and if the same hybrids perform better compared to their parents when feeding on food none of the species are adapted to. We generated two first-generation hybrid crosses between species of African cichlids. In feeding efficiency experiments we measured the performance of hybrids and parental species on food types representing both parental species niches and additional ‘novel’ niches, not utilized by either of the parental species but by other species in the African cichlid radiations. We found that hybrids can have higher feeding efficiencies on the ‘novel’ food types but typically have lower efficiencies on parental food types when compared to parental species. This suggests that hybridization can generate functional variation that can be of ecological relevance allowing the access to resources outside of either parental species niche. Hence, we provide support for the hypothesis of ecological context-dependency of the evolutionary impact of interspecific hybridization.

## **Introduction**

Adaptive radiation is the process whereby an ancestral lineage rapidly diversifies into multiple phenotypically and ecologically differentiated species (1). This process is thought to be driven by a combination of availability of otherwise underutilized resources (i.e. ecological opportunity), intraspecific competition and divergent natural selection between niches (2). It is often associated with colonization of a novel adaptive zone, the extinction of previous

tenants of an existing adaptive zone, or the evolution of a key innovation that provides access to a previously inaccessible adaptive zone, all of which create ecological opportunity (3).

However, only some lineages actually respond to these favourable conditions and diversify, while most lineages seem to remain indifferent (1). Rapid expansion into and adaptation to a multitude of new niches, as is characteristic in large adaptive radiations (4), requires a large amount of heritable phenotypic variation that is ecologically relevant (5). One mechanism that can quickly generate high levels of such heritable variation is interspecific hybridization (6,7,8,9). Hybridization can generate novel phenotypes through the combination of alleles that have not segregated before in the same population (10,11,12,13) and may release populations from constraining genetic correlations (14), thereby increasing evolvability (15,16,17,18) and theoretically facilitating adaptive peak shifts (19). Unless intrinsic incompatibilities are very strong, such ecological advantages of interspecific hybrids may, at times of ecological opportunity, well outstrip the negative effects associated with intrinsic incompatibilities (20,21,22).

Hybridization is indeed a common feature in adaptive radiations (13,20). Some entire radiations started from a hybrid swarm (e.g. Hawaiian Silversword plants: 23 / Cichlid fish: 24), and recurrent hybridization among constituent species is known from many radiations and has been invoked as a facilitator of diversification (e.g. Shrubs on Lord Howe Island: 25 / Darwin finches: 26 / cichlid fish: 27,28 / Heliconius butterflies: 29). Introgressive hybridization can facilitate adaptation and evolutionary success of existing species by increasing their potential for range expansion and rapid adaptation to new or changing environments, and may in this way indirectly trigger diversification in adaptive radiation (30). Hybridization may also contribute more directly to the build-up of species diversity during adaptive radiations (and elsewhere) if hybrid lineages can establish and persist ecologically and genetically as new species alongside the parental species (9,21,22,31,32). If performance trade-offs are associated with the respective adaptations of two species, their hybrids are expected to be intermediate in performance and to do less well than do individuals of either parental species in their respective niches. This ecological hybrid performance disadvantage or ecological incompatibility hypothesis is central to ecological speciation theory (1,8,33) and supported by a number of experimental studies (e.g. fish 34-36; insects: 37)).

These apparently contradictory predictions for ecological hybrid performance may be fully compatible and complementary if the influence of hybridization on evolution was dependent on the ecological context, i.e. whether the hybrids have access to ecological opportunities outside the parental species niches (7,8,9). Surprisingly, these two sets of predictions for animals have to the best of our knowledge never been tested in the same experiment. Here we set out to do so.

By measuring feeding performance of cichlid fish species hybrids and the corresponding non-hybrids in different ecological contexts, we extend the ecological speciation testing framework. We do not just test the prediction of functional hybrid incompatibility, i.e. ecological hybrid performance disadvantage in parental species niches, but also at the same time the prediction of an ecological hybrid performance advantage in 'novel' niches. We test the same first-generation hybrid and non-hybrid individuals on food

types representing the niches of their parental species and - depending on the parental species that were crossed - on 'novel' food types that neither of the parental species is adapted to. We refer to them as 'novel', but whereas one is truly novel to the entire cichlid radiation, we chose the other food types to represent prey types that are rare in the diet of the species that we test here, but that provide the resource basis for entire trophic guilds of other cichlid species in each of the large African lakes (38,39).

To test the ecological context-dependency of interspecific hybridization we experimentally test the same first-generation hybrids and non-hybrids on food types representing the niches of the parental species as well as on 'novel' food types. This results in three main predictions:

1. The parental species show tradeoffs between feeding effectively on the food type they are adapted to and on that of the other species, such that each species performs best on the food type that resembles its natural diet.
2. When tested on the parental species resources, hybrid performance is intermediate between that of both parents on both food types, leading to ecological hybrid performance disadvantage.
3. When tested on 'novel' food types, hybrid performance often exceeds that of parental species, resulting in ecological hybrid advantage.

All tests were done with fish raised in a common garden environment. We chose two different ecological specialists from the Lake Victoria radiation, the reef zooplanktivore *Pundamilia* sp. 'nyererei-like' (28) and the epilithic algae scraper *Neochromis omnicaeruleus* (40). These species differ profoundly in morphology and ecology (41) but are only about 15'000 years divergent (24). We crossed the same reef zooplanktivore from Lake Victoria also with an omnivorous generalist species from Lake Malawi (*Astatotilapia calliptera* (42)). These species are about 2.74 to 4.82 million years divergent (43). The food types used in the feeding efficiency experiments resembled the food types that the parental species specialize on (i.e. either evasive zooplankton (copepods) or epilithic algae), and other food types (i.e. gammarids and shrimps) that are completely absent from African lakes (gammarids) or are the main food of other species and entire guilds of cichlids in the same radiations (shrimps).

## Material and Methods

### *Parental species and their first-generation hybrids*

All fish used to generate the first-generation hybrid crosses and used in the experiments derived from laboratory populations bred and raised under identical conditions in the research aquarium system at EAWAG. These populations descend from wild individuals caught in Lake Victoria from the islands of Makobe (*N. omnicaeruleus*) and Python (*P. sp. 'nyererei-like'*) and in Lake Malawi from the island of Chizumulu (*A. calliptera*) (>5 generations in the laboratory). We will refer to these three species and the two first-generation hybrid crosses as NO for *N. omnicaeruleus*, PN for *P. sp. 'nyererei-like'* and AC for *A. calliptera* and we

use the corresponding acronyms for the hybrid crosses, as follows: mother species x father species, i.e. ACxPN and PNxNO.

The crosses differ in the genetic distance and in morphological features between the parental species (for details see Supporting Information Appendix 1 and 2). We obtained six independent full-sib first-generation hybrid families of PNxNO and two half-sib (same father) first-generation hybrid families of ACxPN from which several individuals of each family were tested. Individuals of the parental species tested in the experiments derived from several single-species stock tanks, each containing a population lab-bred for multiple generations. To avoid any tank or population effects we tested individuals from two or three single species stock tanks of *N. omnicaeruleus*, *A. calliptera* and *P. sp. 'nyererei-like'*. All fish were raised in a common garden environment (for details on the breeding and rearing of the parental species and their first-generation hybrids see Supporting Information Appendix 3).

### *Experimental set-up*

All individuals used in the experiments were adult males at the age of ten to seventeen months. Females were not tested to avoid confounding effects of sexual dimorphism that may influence feeding efficiency in cichlids (44). The fish were tested in groups of 7 to 16 fish in individual experimental tanks after an acclimatization period. At the end of the acclimatization period the fish were fed for habituation on 4 consecutive days each once with one of the four food types (see below) used in subsequent experiments. Prior to the exposure to the different food types the parental species and the hybrid crosses were naïve to the presented food types. Two days after the acclimatization phase the alternating experimental schedule started, consisting of one day testing with one of the food types, followed by one day where the fish were not fed. This experimental procedure was continued with each food type (trials with three different food types: algal substitute, gammarids and shrimp) until a sufficient number of successful feeding trials for each individual fish of an entire group had been collected on all three food types. We aimed at getting several successful feeding trials to account for individual variation in feeding efficiency. All fish in a group were tested together to be able to account for possible group effects (see below). Finally, each fish was tested on zooplankton and after one successful trial the fish was sacrificed in accordance with Swiss animal experimentation permits. All fish within an experimental group were tested in the same order on the different food types, and the order alternated between different experimental groups (table S1; for details on the experimental setup see Supporting Information Appendix 4). Altogether a total of fifty-six fish in 5 experimental groups were successfully tested on one, several or all food types (table S1). All experimental feeding trials were videotaped and analysed afterwards.

### *Food types*

We used four different food types in individual feeding trials. Two food types resemble the main diet that two of the parental species are specialized on: an algal substitute to resemble firmly attached epilithic algae, the dominant diet of the epilithic algae scraper *N. omnicaeruleus* and copepods resembling the dominant zooplankton taxon found in Lake

Victoria and the main diet of the reef zooplanktivore *P. sp. 'nyererei-like'* (40,45,46). Furthermore, one of the previously mentioned food types (epilithic algae) and two other food types were 'novel' in the sense that - depending on the parental species that were crossed - none of the parental species are adapted to. Although we refer to them as 'novel', only one is truly novel to the entire cichlid radiation (gammarids), whereas the other food types (shrimps and algal substitute) represent prey types that are very rare in the diet of all of the test species except the algal substitute for *N. omnicaeruleus*, but that entire trophic guilds of other cichlid species in each of the large African lakes have adapted to (38,39). For the cross involving the reef zooplanktivore *P. sp. 'nyererei-like'* and the omnivorous generalist species *A. calliptera* the algal substitute is a 'novel' food type both for the parental species and the hybrids. We chose gammarids to resemble slow moving but powerful evasive benthic prey and shrimps to resemble powerful burst-swimming pelagic prey. The percentage of different food types in the diet of the omnivorous generalist species *A. calliptera* is not known. Details on the preparation and testing of each food type can be found in the Supporting Information Appendix 5.

#### Statistical analysis

All analyses were conducted using R statistical software, version 3.5.1 (47). When normality assumptions were satisfied (Shapiro–Wilk test), parametric statistics were used to analyse the data, otherwise non parametric tests were applied. In all linear mixed models we checked assumptions of normality for the residuals using residual versus predicted plots and normal probability plots. *P*-values for all post hoc tests were adjusted for multiple comparisons using a false discovery rate of  $\alpha = 0.05$  (48; hereafter FDR). We report both raw and FDR-adjusted *p*-values for the linear mixed models in the tables present in the Supporting Information but only the FDR-controlled *p*-values in the text.

All data are available from the Dryad Digital Repository:  
<https://doi.org/10.5061/dryad.m25t886>.

#### *Species differences in feeding efficiency*

Feeding efficiency was estimated as the ratio between the amount of consumed food types (weight difference for the algal substitute and number of prey types for gammarids, shrimps or zooplankton) and the total number of feeding bouts (number of unsuccessful and successful attacks). We tested if differences exist between the parental species and their hybrids in the feeding efficiencies on each food type. For food types other than zooplankton, we had repeated measures of an individual's feeding performance; in the majority of cases we obtained an individual's feeding performance from three successful experimental trials. In a few cases this number deviated (from 1 to 5), see table S2) and some individuals were either not tested on all food types or they did not feed on one or several food types.

Feeding efficiency was analysed using linear mixed effect models with the package lme4 in R (49). The feeding efficiency model included the fixed effects "Group" (the three parental species and both first-generation hybrids were each classified as a group), "BS" (=body size measured as standard length) and "Trial length" (the duration of an experimental

trial). Separate tests of feeding efficiency on the four food types were run for each hybrid cross and their parental species (PN/NO/PNxNO and AC/PN/ACxPN). Each model included the random factors “Individual” (used only in the models where individual fish were tested repeatedly: algal substitute, gammarids and shrimps), and “Experimental batch”. The latter accounts for a possible batch-effect as individual fish were tested in different experimental batches. Since each batch was tested over a duration of 2-6 months and at a different time of the year, each group of fish may have experienced slightly different experimental conditions. Furthermore, due to experimental constraints the trial length varied in a few cases significantly between groups tested on several food types, which could influence feeding efficiency as some individual fish were exposed longer to the different experimental treatments. We allowed for an interaction between “Group” and “BS” to account for the interdependence of the size of the fish and feeding efficiency in each group, as was revealed by preliminary linear models.

To assess the impact of the predictor variables (i.e. the relative-impact value; hereafter RI) in the model we used an AIC-based model averaging approach (50). The full set of additive models was generated and then the relative importance of each predictor variable was calculated. This can vary on a scale from 0 to 1 and is calculated as the sum of the Akaike weights of the models in which the variable appears. Better models have larger Akaike weights and thus a variable that contributes more to model fit will have a higher relative-importance value (i.e. closer to one). We calculated the differences in feeding efficiency between groups based on the least-squares means and confidence intervals extracted from the feeding efficiency model. For assessing the fit of the model the root mean square error (RMSE) between the predicted and observed values and the AIC were calculated. Normality assumptions were not met in any of the linear mixed models. Hence, we log-transformed the response variable to achieve a fit to a normal error distribution.

#### *Differences in size, weight, trial length, latency and possible trial learning effect*

We tested several parameters that may influence feeding efficiency (e.g. allometric effects of size on feeding efficiency) between the parental species and hybrid crosses as well as two behavioural parameters, latency and learning (details are provided in the supporting information Appendix 6). In general, the parental species and the hybrid crosses did not differ in size or weight when tested for each food type separately with one exception (table S3). There were more cases where the trial length between parental species and their hybrid cross differed when tested for each food type (table S4). Due to some significant differences in size, weight or trial length between the parental species and their hybrid cross, we used the parameters as fixed effects in the linear mixed model. Since size and weight differences were highly correlated and body size can contribute to the ability of utilizing specific food types (51) we only accounted for size as a fixed factor in the model. We further looked at two behavioural parameters, latency to first-feeding and a possible learning effect due to repeated testing of specimens on the four food types.

## **Results**

## Differences in feeding efficiency between parental species and first-generation hybrids

Parental species and the first-generation hybrids differed significantly in feeding efficiency on several of the different food types, with “Group” being the best predictor of feeding efficiency in all models with one exception (RI value across all food types ranged between 0.43 and 1; table S5).

Corroborating our first prediction we found adaptational trade-offs in feeding efficiency between the two specialists, the epilithic algae scraper *N. omnicaeruleus* and the reef zooplanktivore *P. sp. ‘nyererei-like’*, on their specific food types. Both specialists were most efficient feeding on the food types that resembled their natural diet (algal substitute: figure 1a,b and zooplankton: figure 1g,h; table S6).

We predicted further that both hybrid crosses would show an intermediate performance on the parental species resources, which would result in an ecological hybrid performance disadvantage in nature if populations were resource limited. Results of our feeding efficiency experiments were largely in agreement with these predictions: When feeding on zooplankton the first-generation hybrid cross ACxPN had a significantly intermediate performance compared to both parental species (figure 1h; table S6). The first-generation hybrid cross PNxNO had a significantly higher performance than the epilithic algae scraper *N. omnicaeruleus* but did not differ from the specialist reef zooplanktivore *P. sp. ‘nyererei-like’* (figure 1g; table S6). When feeding on algal substitute, the first-generation hybrid cross PNxNO was intermediate in performance compared to both parental species (figure 1a; table S6).

Finally, in agreement with our third prediction when tested on ‘novel’ food types - whereby the novelty of the food type depended on the parental species that were crossed - both hybrid crosses outperformed their parental species each on one ‘novel’ food type or were as efficient as the best-performing parental species. In the experiments involving the first-generation hybrid cross PNxNO and the respective parental species two out of the four food types were ‘novel’, namely shrimps and gammarids. When feeding on shrimps the PNxNO hybrids significantly outperformed both parental species (figure 1e; table S6) and when feeding on gammarids both the epilithic algae scraper *N. omnicaeruleus* and the hybrids significantly outperformed the specialist reef zooplanktivore *P. sp. ‘nyererei-like’* (figure 1c; table S6). In the experiments involving the other first-generation hybrid cross ACxPN and the respective parental species, three out of the four food types were ‘novel’, namely shrimps, gammarids and algae: When feeding on algae, the hybrids significantly outperformed both parental species (figure 1b; table S6) and when feeding on shrimps both the omnivorous generalist *A. calliptera* and the hybrids showed a weak trend towards outperforming the specialist reef zooplanktivore *P. sp. ‘nyererei-like’* (figure 1f; table S6). When feeding on gammarids none of the three groups (parental species or their first-generation hybrids) differed significantly in their performance (figure 1d; table S6). Finally, the ecological generalist *A. calliptera* exhibited no superiority on any of the tested food types (figure 1b,d,f,h; table S6).

In three cases body size and group (i.e. cross type and parental species) had a similar relative-impact value on feeding efficiency (table S5). Body size and group both had large

relative impact values in the AC/PN/ACxPN dataset on the efficiency in feeding on algal substitute (“Group” RI=0.63; “BS” RI=0.41) and in both datasets on the efficiency in feeding on gammarids (PN/NO/PNxNO: “Group” RI = 0.43, “BS” RI= 0.78; AC/PN/ACxPN: “Group” RI= 0.63, “BS” RI= 0.38). In the AC/PN/ACxPN dataset on the algal substitute we found that larger fish (linear regression;  $r=0.33$ ,  $P=0.008$ ) and particularly larger fish of *A. calliptera* ( $r=0.69$ ,  $p=0.002$ ) had a higher feeding efficiency on the algal substitute. When feeding on gammarids larger fish in general (independent of species or cross identity) ( $r=0.40$ ,  $p<0.001$ ) and larger individuals of the epilithic algae scraper *N. omnicaeruleus* ( $r=0.73$ ,  $p<0.001$ ) were significantly more efficient. Similarly, in AC/PN/ACxPN experiments larger fish (independent of species or cross identity) were significantly more efficient when feeding on gammarids ( $r=0.30$   $p=0.011$ ). Finally, although body size was not a strong predictor of feeding efficiency on zooplankton in the AC/PN/ACxPN experiments, there was a significant interaction between body size and group (“Group\*BS”  $p=0.009$ , table S5). This was driven by a significant negative relationship between body size and feeding efficiency on zooplankton in the hybrids ( $r=-0.71$ ,  $p=0.021$ ) and the opposite relationship was observed in the parental species *A. calliptera* ( $r=0.81$ ,  $p=0.015$ ).

We further looked at two behavioural parameters, latency and learning. In most cases the parental species and the hybrids did not differ in their latency (i.e. the time in seconds it took an individual to first attack a prey item (e.g. zooplankton, gammarids or shrimps) or take a first bite of the algal substitute). Yet, in two cases the generalist parental species *A. calliptera* showed a significantly lower latency than the hybrid cross ACxPN or the parental species *P. sp. ‘nyererei-like’* in a first attack on a shrimps or taking a first bite of the algal substitute. Also, in one case the hybrid cross PNxNO showed a significantly lower latency than then parental species *N. omnicaeruleus* in a first attack on shrimps (table S7). Finally, no learning effect was observed on any of the four food types, neither for all individuals of the parental species combined nor for all individuals of the hybrid crosses combined, nor when all individuals were combined into one analysis (table S8).

## Discussion

Interspecific hybridization can have a variety of consequences for the evolutionary process, including the erosion of existing adaptation or allowing for rapid adaptation to a new situation, constraining or promoting speciation, or leading to the reversal of speciation and loss of species distinctiveness well after speciation (9). The outcome of hybridization depends on the interactions between the intrinsic viability of the hybrids, the ecological environment including the presence of other species, and on the fitness of hybrids in that ecological context (9). Theoretical models predict that the ecological performance of hybrids is context-dependent, such that the propensity of hybrids to show ecological performance advantage or disadvantage is largely determined by the ecological context in which hybridization happens (19,31,52; but see 53 and 54 for non-ecological mechanism). These models predict that ecological hybrid performance advantage requires ecological opportunity beyond the parental species’ niches and strong ecological constraint, excluding the parental species from the habitats or resources that lie outside their niche (19,31,52). This would then facilitate the opportunity for range expansion of hybrids, rapid adaptation to new or changing



environments and the invasion of new niches and thereby facilitate the process of adaptive radiation (13,19,20). Many studies of natural hybridization in animals and plants found that natural hybrids are ecologically distinct from their progenitors (e.g. 27,55-60). In several plants the ecological divergence of natural hybrids from the parental species is indeed due to the expression of novel ecological functions (60,61), which can cause a fitness advantage of the natural hybrids in the novel ecological niche when compared to their progenitors (61-63). Experiments with experimental hybrids, even just first-generation hybrids as in our case, help to elucidate if hybridization can initially generate novel functions that are ecologically relevant, a prerequisite for hybrids to possibly escape competition from their progenitors by occupying a novel ecological niche (19,62-65).

Here, we investigated the ecological context-dependency of the consequences of hybridization on individual feeding performance by experimentally testing first-generation hybrids and parental species on food types representing the niches of the parental species as well as on 'novel' food types. We found that - consistent with our first prediction and with ecological speciation theory (1,8,33) - the two ecological specialist parental species, the reef zooplanktivore *P. sp. 'nyererei-like'* and the epilithic algae scraper *N. omnicaruleus*, both performed best on the food types that resemble their natural diet, zooplankton and epilithic algae, respectively. The third species, the omnivorous *A. calliptera*, was an ecological generalist, and it showed no superiority on any of the tested food types over either of the other species. Our second prediction, ecological hybrid performance disadvantage when feeding on the parental species resources, was also largely met: In two out of three cases the hybrids did show an intermediate performance on both types of parental species resources, i.e. less good than the specialist. In the third case the hybrids performed equally well as the specialist parental species. Finally, consistent with our third prediction both hybrid crosses outperformed both parental species on a 'novel' food type, and were as efficient as the best-performing parental species on the other 'novel' food types.

To the best of our knowledge, this is the first experiment in animals that tested and demonstrated ecological context-dependency of ecological performance of interspecific hybrids and their parents. In natural situations where ecological opportunity outside of the parental species niches exists that hybrids with novel phenotypes can tap into, effects of hybridization on diversification can be twofold. First, it may have a direct effect on diversification and speciation (e.g. in adaptive radiations) by instantaneously generating a highly evolvable hybrid population and thereby facilitating peak shifts potentially resulting in the establishment, stabilization and persistence of a hybrid species alongside the parental species (26). Second, it may indirectly facilitate adaptive radiation by allowing an existing species to rapidly adapt to a changing environment and hence prevent extinction, or to widen its niche and expand its range (66). Our results also illustrate the importance to explore the functional performance of hybrids in multiple ecological contexts, not just those the parents are adapted to, to better understand the ecological and evolutionary impact of interspecific hybridization.

Our experimental data imply that first-generation hybrids may tap into resources, and hence access potential niche space that is inaccessible for the parental species. We do not

know the functional mechanism nor its genetic basis. Heterosis or hybrid vigour could possibly explain part of the feeding efficiency of our first-generation hybrid crosses. Heterosis is only present in first-generation hybrids and has therefore no adaptive significance in subsequent generations (65,78). Heterosis can affect many different traits, e.g. physiology (thermal tolerance (65)) or behaviour (boldness/shyness (78)) and its magnitude and outcome (i.e. positive or negative) is dependent on the ecological context and the strength of the ecological contrast tested (65, 78). However, heterosis is unlikely to explain the experimental support for our key directional predictions of context-dependence that we present here, namely that hybrids be intermediate in performance between parental species in parental niches but to outperform both parental species in novel niches.

The adaptive radiations of African cichlids are known for their diverse ecological specializations, which are accompanied by a suite of morphological and behavioural traits that show a complex interplay with feeding performance (67). These include, for example, ram and suction feeding behaviours (68), body-angle orientation when feeding on epilithic algae (69), the extent of hypertrophied lips (70), variation in tooth morphology (71), the use of vision and/or the cephalic sensory system for prey detection (72) or biting/suction-related differences in head and mouth morphology (46,73). These empirical and experimental studies suggest that not a single trait but a combination of traits (e.g. locomotor behaviour, sensory system and functional morphology) underlies feeding performance. Furthermore, individual components of morphology and performance can be decoupled, such that different combination of traits may confer the same function (74).

Experimental and experimentally informed simulation studies have shown that hybridization in cichlids can produce functional novelty when there is a many-to-one mapping of form to function (75,76). This, together with findings that a part of the morphological variation and the major phenotypic axes of first-generation hybrids are retained in later-generation hybrids (17), make it likely that the superior feeding efficiency on the 'novel' food types that we document in our first-generation hybrids will be present to some degree also in a fraction of later-generation hybrids. To determine how morphology interrelates with feeding performance-related behaviour in hybrids an integrative approach studying the relationship between functional morphology, behaviour and performance is needed. Such an experiment should ideally be extended to second-generation hybrids.

Another promising direction of research would be experimental evolution in large mesocosms conducted over multiple generations to measure fitness related traits such as growth or survival in hybrids and the parental species (34,36). Albeit many studies in fish have used feeding efficiency as a proxy for performance and fitness in the context of ecological speciation and have showed that morphology can be linked to function, ecological feeding specialization and thus ecological niche (68-70,73), we acknowledge that feeding efficiency captures only one of many possible components of fitness (8) and that other proxies such as growth or survival may more conclusively demonstrate the successful exploitation of an ecological niche because they integrate a larger number of components. Multi-generation hybrid fitness experiments on multiple ecological niches could be particularly interesting if they involved experimental hybrids between ancestral species of adaptive radiations with

hybridogenic origins (13,24,77) or between constituent species from adaptive radiations for which recurrent hybridization has been invoked as a facilitator of diversification (25-28). Multi-generation experiments would also allow to partition fitness variation into effects of heterosis versus novelty. Furthermore, hybrid crosses from a broader phylogenetic scope of parental species (11) and more ecological contrasts than used in our study would be desirable to be able to draw conclusions about the generality of ecological hybrid advantage as a factor in the diversification process. Together with dense QTL-mapping or genome-wide association mapping, the genes or gene complexes with phenotypic effects can be detected (13). These can be compared to the genes from different parental species which segregate in a hybrid populations mosaic genome and may help to assess how the sorting of these segregating genes or gene complexes contribute to adaptive radiations (13,24,77). Hybridization results in the introduction and reassembling of genetic variation that can both have deleterious or beneficial intrinsic or extrinsic postzygotic effects, and comparative experimental studies are needed to assess how these different effects of hybridization have shaped the genomic architecture of ecology relevant traits in groups of hybridizing species (9,13,22). Experiments over multiple generations that further vary the strength of the ecological contrast that is being tested and thereby mimic natural systems with fluctuating adaptive landscapes will allow to test how important extrinsic postzygotic isolation is in the speciation process (33-35, 80).

Earlier studies on experimental first- and second-generation cichlid hybrids, with similar divergence times between the parental species as in our study, have revealed that hybrids are viable and fertile into higher generations between species several million years divergent (43,81). Some of these hybrids regularly exhibit novel phenotypes in colour and morphology when compared to their parental species (15,17,43,75,82). Several of these studies have further suggested that the observed novelties may be co-opted by natural (or sexual) selection since many of the experimental hybrids have been shown to resemble other species known from the cichlid radiations. Also, patterns of morphological diversity found in these hybrids have been shown to predict those observed in extant cichlid radiations (15,17,83).

In conclusion, the data we presented here constitute experimental evidence, lacking so far (56), that functional novelty in cichlid hybrids can be of adaptive relevance in certain ecological contexts and thereby possibly facilitate niche shifts, promote adaptive diversification and - when retained and stabilized in later-generation hybrids - speciation. We provide support for the hypothesis of ecological context-dependency of the evolutionary impacts of interspecific hybridization. Our findings corroborate recent theoretical work (19) that in environments with many different and accessible ecological niches, of which only a subset is occupied, transgressive morphology (in our case functional traits) in hybrids can facilitate the process of adaptive radiation through ecological hybrid performance advantage. We suggest that this process was important for the diversification of cichlids of the great lakes of Africa.

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#### **Author contributions:**

OMS and OS conceived the experiment, OMS designed and performed the experiments and processed and analysed the data and OMS and OS wrote the manuscript together.

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**Figure 1**

Feeding efficiency of two ecological specialists and one generalist cichlid species and their first-generation hybrids reveals an ecological hybrid performance advantage over the parental species when feeding on 'novel' food types as well as divergent feeding efficiencies of the two ecological specialists between the food types they are adapted to. Feeding efficiency was measured on four food types; (a,b) algal substitute, (b,c) gammarids, (e,f) shrimps and (g,h) zooplankton. The species *P. sp. 'nyererei-like'* (PN) and *N. omnicaeruleus* (NO) are ecological specialists (i.e. reef zooplanktivore and epilithic algae scraper, respectively) and *A. calliptera* (AC) is a generalist omnivore. The feeding efficiency on the respective food types that the parental species are adapted to are highlighted in green for *N.omnicaeruleus* and in red for *P. sp. 'nyererei-like'* and highlighted in orange are the food types where the hybrid crosses showed a higher feeding efficiency than either parental species. Given on the y-axis are the mean and the standard deviation per group (species or hybrid cross). Feeding efficiency (FE) for each individual was estimated as the ratio between the amount of consumed food types (weight difference for the algal substitute and number of prey types for gammarids, shrimps or zooplankton) and the total number of feeding bouts (number of unsuccessful and successful attacks). The number of individuals tested on each food type for each group is given at the bottom of each graph. The individuals were each tested multiple times for all food types except for zooplankton. The differences in feeding efficiency between groups were calculated from the least-squares means and confidence intervals of each group extracted from a linear mixed feeding efficiency model (table S6). *P*-values were adjusted for multiple comparison with the false discovery rate (FDR) method; •*p* < 0.1, \**p* < 0.05, \*\**p* < 0.01 and \*\*\**p* < 0.001 (See table S6 for details on raw and FDR-adjusted *p*-values).

