Supporting information

Interspecific hybridization can generate functional novelty in cichlid fish

Appendix 1: The ecology of the parental species used to create the first-generation hybrid crosses

The *Astatotilapia calliptera* population used in our study comes from Chizumulu Island, an offshore island in the middle of Lake Malawi. The two Lake Victoria species are from the southern part of the lake; the population of *Pundamilia sp. 'nyererei-like'* comes from Python Island, situated in the Mwanza Gulf. The population of *Neochromis omnicaeruleus* comes from the offshore island of Makobe. The three species differ in their ecology and morphology.

Astatotilapia calliptera (Günther 1894) has a generalized morphology and is an omnivorous cichlid that feeds on a variety of food types and its main diet likely consist of loosely attached benthic prey and algae (Konings, 2007). No data is available on the specific diet components or the feeding behaviour of *Astatotilapia calliptera*.

Pundamilia sp. 'nyererei-like' is superficially similar in morphology to *Astatotilapia calliptera*. It has a similarly straight to concave dorsal head profile, a terminal to superior positioned mouth, a slightly less wide jaw than *Astatotilapia calliptera* with similar dentition, such that the outer tooth row is widely spaced with unicuspid teeth and the few inner tooth rows consist of unequally bicuspid to unicuspid teeth (Greenwood, 1979; Witte-Maas & Witte, 1985). *Pundamilia sp. 'nyererei-like'* is specialized to feed on zooplankton; the yearly average percentage of zooplankton in the diet of *Pundamilia sp. 'nyererei-like'* (Meier et al., 2016) from Python island is approximately 50% with the rest of the diet consisting of loose benthic prey (Bouton et al., 1997). All of these different diets require feeding techniques that involve suction (Barel, 1983; Wainwright et al., 2001; Hulsey & García de León, 2005) and the two major feeding behaviours of *Pundamilia sp. 'nyererei-like'* in the wild are snapping and picking; to a much lesser extent they also use pulling and pull-scraping behaviours (Seehausen & Bouton, 1998).

Neochromis omnicaeruleus has a typical algae scraper morphology (Barel, 1983; Bouton et al., 2002; Rupp & Hulsey, 2014), with a decurved dorsal head profile, a subterminal mouth, a short and wide lower jaw with many closely spaced bicuspid teeth in the outer rows and with many inner tooth rows holding tricuspid teeth (Seehausen et al., 1998). The yearly average percentage of filamentous algae in the diet of *Neochromis omnicaeruleus* from Makobe Island is approximately 80% with the rest of the diet consisting of insect larvae (Bouton et al., 1997; Seehausen & Bouton, 1998). A filamentous algae diet requires feeding techniques that involve biting (Bouton et al., 1999, 2002). The two major feeding behaviours of *Neochromis omnicaeruleus* in the wild are pulling and pull-scraping and to a much lesser extent they also show snapping and picking behaviours (Seehausen & Bouton, 1998).

Appendix 2: Genetic distances of the parental species used to create the first-generation hybrid crosses

One of the hybrid crosses represents an intra-radiation cross (PNxNO, both parental species are from Lake Victoria) and the other an inter-radiation cross (ACxPN, the parental species are from Lake Malawi and Lake Victoria). The crosses differ in the genetic distance and in morphological features (see Appendix 1) between the parental species. The uncorrected p-distances (genetic distances) are calculated from mitochondrial D-loop sequences (Stelkens et al., 2009). The genetic distance for the cross PNxNO is not given in Stelkens et al. (2009). However the genetic distance between *N. omnicaeruleus* and *Pundamilia sp. "pundamilia-like",* the sister species to *Pundamilia sp. 'nyererei-like'* was calculated and the divergence time should thus be similar to that of the cross PNxNO. Hence, for the cross PNxNO the genetic distance between the parental species is approximately 0.007 and for the cross ACxPN it is 0.055. Stelkens et al. (2009) used three different molecular clocks, which translate the genetic distance into a range of divergence time (i.e. absolute time since speciation) from 0.35 to 4.82 million years (internal/fossil record/Gondwana fragmentation calibration). Depending on which molecular clock is used the genetic distance of the hybrid PNxNO translates into a divergence time of 0.35 to 0.61 million years and that of the hybrid ACxPN into a divergence time of between 2.74 to 4.82 million years (Stelkens et al., 2009).

Appendix 3: Breeding and rearing of parental species and first-generation hybrid crosses

Both first-generation hybrid cross families were obtained by keeping between 5-20 females of one species together with one heterospecific male. The individuals of the parental species used to create the hybrid crosses each derived from single-species stock tanks containing lab-bred population of multiple generations. Five days after spawning the fertilized eggs were removed from the mouth of the mother and transferred to, and raised in identical egg tumblers for 14 days. The fry were subsequently moved to small aquaria (20x40x20 cm), and after 1-2 months they were transferred to larger aquaria (100x40x30 or 200x40x30 cm) where they stayed until the start of the experiments. Each family was raised in a separate aquarium. The majority of aquaria holding the hybrid crosses and parental species were part of a large recirculation system. Some additional tanks were stand-alone with internal filtration (two and one tanks holding PNxNO and ACxPN hybrids, respectively). Both aquarium types had water temperature at 24±1°C and a 12:12 h light/dark cycle. All fish were fed the same diet ad libitum. Twice a week all tanks were fed a mixture of ground shrimps and peas, enriched with spirulina powder, and on the other days with commercial cichlid flakes. All the housing tanks of the parental species and first-generation hybrid crosses harboured similar densities of fish.

Appendix 4: Experimental set-up

For the experiments we used thirty-two 25 liter tanks (25x25x40 cm), each equipped with an internal filter and situated in the same experimental room with the same light and temperature

conditions (water temperature at 24±1°C and a 12:12 h light/dark cycle using fluorescent tubes). Once a week one third of the water was exchanged, and the internal filters were cleaned once a month. Experiments were performed from December 2012 until April 2014. The experimental setup consisted of two rows of sixteen tanks, alternating within each row between experimental and non-experimental tanks. The non-experimental tanks harboured a female of an unrelated species to ensure that the test fish would not be deprived of social contacts throughout the experimental period (which sometimes lasted for up to four months). Hence eight experimental tanks could be used in each row of tanks. The experimental tanks were sealed off with blue foil at the back and at one side adjacent to the neighbouring aquaria so that the test fish would only see one neighbouring female fish. The tank floor was covered with a thin layer of sand.

Only males were used for the experiments to avoid confounding effects of sexual dimorphism that may influence feeding efficiency in fish (Tkint et al., 2012; McGee & Wainwright, 2013). Fish were placed in the tanks one and a half weeks prior to the start of an experimental trial to allow acclimatization and additionally to familiarize the fish with the four different experimental food types. After acclimatizing for two days, during which the fish were fed commercial cichlid pellets, we fed on consecutive days ten living shrimps, ten living gammarids, a standardized amount of living zooplankton, and an algal substitute (see description below for each food type). After the 4 days of feeding on the experimental food types the fish were fed commercial cichlid pellets for another two days, followed by a day without food. Following these acclimatization days the experimental schedule started, consisting of one day testing with one food type, followed by one day of no food. During the weekends fish were not tested. They were fed on Saturdays with commercial cichlid pellets and not fed on Sundays. On an experimental day the internal filter was removed early in the morning and returned after the feeding trials, which always started in the afternoon, were finished. If a fish on an experimental day did not feed on the food type it was given a small amount of commercial cichlid flakes instead. Up to sixteen fish were tested in the same experimental batch, which contained a mix of individuals from at least two different groups (parental species or hybrid cross; Table S1). All experimental feeding trials were videotaped with either a 50mm macro lens (Tokina; Machida, Tokio, Japan) mounted on a Canon 60D camera (Canon; Ota, Tokio, Japan) or with a surveillance camera system (V-security, Leipzig, Germany). Once all fish of one experimental batch had been successfully tested, we exchanged them for a new experimental batch of fish. Standardized photos of the fish were taken with a Canon 60D camera and a 50mm macro lens before their introduction into the experimental tanks. Furthermore, the weight (to the nearest 0.01 gram) and size (standard length, to the nearest 0.01cm) of each fish was taken prior to the start and after the termination of the experiments. Means of length and weight were used for subsequent analyses. The aim was to test each fish at least three times on each food type except for zooplankton, because sacrificing of the fish was necessary after the zooplankton trial in order to ascertain the number of ingested zooplankton.

Appendix 5: Preparation and testing of each food type

Algal substitute

We used adapted protocols of Bouton et al. (1998) and Parsons et al. (2014) to create algae substitutes that mimic filamentous algae. Algal substitutes were prepared by mixing 1.7 grams of Agar with 10 ml of water and 10 grams of a mixture of ground shrimps, peas and *Spirulina* powder. This concoction was heated in the microwave at 1000 watts for 15 seconds and then immediately spread out on a petri dish with a diameter of 5.7 cm. The petri dish had a plastic mesh glued to the bottom, so that the hot concoction filled the holes of the grid and thereby produced a firmly attached 0.8 cm layer of algal substitute. The excess of algal substitute on top of the petri dish was removed to make a homogenous flat top layer and then left to dry at room temperature for thirty minutes. Afterwards the petri dishes were soaked in water for twenty-four hours to become completely saturated. In each trial, the weight (to the nearest 0.001 gram) of the algal substitute was measured twice prior to and after an experimental trial. Before weighing, we dried the sides and bottom of the petri dish with a towel and removed surface moisture by putting a towel on top of the petri dish for five seconds. To estimate the measurement error all the petri dishes from unsuccessful feeding trials (where fish did not feed) were also weighed after the experimental trial. We took the mean weight from the two measures (before and after an experimental trial) to calculate the difference in weight (i.e. measurement error in the case where fish did not feed). We calculated the percentage of weight difference by dividing the difference in weight from before and after the trial by the mean weight from both measurements. The mean percentage in weight difference for algal substitutes calculated from the unsuccessful feeding trials (N=108) was lower than 2%, which indicates that the measurement error was low. The mean difference in weight in the unsuccessful feeding trials (N=108) was 0.009±0.015 (standard deviation) grams, well below the average difference of 0.144±0.178 grams in all successful feeding trials (N=118). An experimental trial started with the introduction of the algal substitute into the front of the tank. A trial lasted for forty minutes after the first feeding attempt. If a fish did not feed within the first hour after introduction the trial was terminated. In preliminary experiments we observed that especially *P. sp. 'nyererei-like'*, *A. calliptera* and ACxPN hybrids fed from the edges of the petri dish, which often resulted in chunks of algal substitute to break loose. To prevent this, we fitted a plastic ring onto the algal substitute resulting in a one-centimetre high wall surrounding the firm layer of algal substitute. This ensured that the fish had to feed from the top with angles between approximately 40-90 degrees against the surface of the algal substitute, and could not feed from the side of the petri dish. Such top-down feeding behaviour is typical for algae scraping species (Rupp & Hulsey, 2014).

Gammarids

Gammarids (Gammarus sp.) were collected in the morning of the experimental day from a little stream at the research station in Kastanienbaum, Switzerland. To acclimatize the gammarids from

the cold stream water (water temperature was approx. between six to eighteen degrees Celsius depending on the time of the year) to the warm aquarium water (24±1°C) we added an air-stone connected to an air pump into the holding tank of the newly collected gammarids and slowly raised the temperature. In the afternoon after acclimatization ten gammarids between five and ten millimeter in length, measured using ten-millimeter grid paper, were randomly picked, photographed with a scale as reference and subsequently released into the front of one experimental tank. A trial lasted for ten minutes after the first feeding attempt. If a fish did not feed within the first ten minutes after introducing the gammarids the trial was terminated. After a successful feeding trial all the remaining gammarids that were not eaten were retrieved and photographed. We measured the size (from the head to the last urosomite) from ten randomly picked sets of ten gammarids that were used in the feeding trials to test if the size range of gammarids that were fed to the fish may differ between days. The mean from these ten sets was 8.4±1.2 millimeter and the sizes of the gammarids ranged between 6.2 and 11 millimeter. Based on a Kruskal-Wallis test the ten sets did not vary in size range ($H=8.79$, $p=0.361$).

Shrimps

The freshwater shrimp species *Neocaridina heteropoda* (Bouvier, 1904), native to East China, was bought from the aquarium trade and grown in a standalone aquarium with temperature and light/dark light regimes matching those used for the fish. The size range obtained in this species and the translucent greenish to brownish coloration resembles that of the freshwater shrimp species *Caridina nilotica* (Roux, 1833), a widely distributed species in African rivers and lakes including Lake Malawi, and the only freshwater shrimp species found in Lake Victoria (Goudswaard et al., 2006). Using a ten-millimeter grid, we randomly collected ten shrimp between five and fifteen millimeter in length, photographed them with a scale as reference, and subsequently released them into the front of the experimental tanks. A trial lasted for ten minutes after the first feeding attempt. If a fish did not feed within the first ten minutes after introducing the shrimp the trial was terminated. After a successful feeding trial all the uneaten shrimp were retrieved and photographed. We measured the size from the eyes to the last segment of the carapax (Goudswaard et al., 2006) from ten randomly picked sets of ten shrimp used in the feeding trials. The mean from these ten sets was 11±1.7 millimeter and the sizes of the shrimps ranged between 7.2 and 17 millimeter. Based on a Kruskal-Wallis test the ten sets did not vary in size range ($H=11.41$, $p=0.180$). The mean and standard deviation from our experiment is similar to the size range of *Caridina nilotica* found in Lake Victoria (Goudswaard et al., 2006).

Zooplankton

Zooplankton was caught early in the morning on the day of the experiment from Lake Lucerne at a depth of five to ten meters using a plankton net (1.2 m in diameter and 250-µm mesh size). The freshly caught zooplankton was first sieved through a 500-µm mesh and - after acclimatization

(see below) - through a 350-µm mesh to obtain a size range of zooplankton between 350 and 500 µm that mostly consisted of copepods, the dominant zooplankton in Lake Victoria (Mwebaza-Ndawula, 1994). To acclimatize the zooplankton coming from cold lake water (approx. six to eighteen degrees Celsius, depending on the time of the year) to the warm aquarium water (24±1°C) we slowly raised the temperature of the water over several hours through aeration with an air-stone. In the afternoon after acclimatization a standard amount of sieved (350-µm sieve) alive zooplankton was taken for the feeding trials with a measuring scoop and suspended in one decilitre of water. The suspended zooplankton was poured into the test aquaria and the feeding trial ran for ten minutes after the first feeding attempt. If a fish did not feed within the first ten minutes after introducing the suspended zooplankton the trial was terminated. After a successful feeding trial the fish was caught and transferred into a new aquarium without zooplankton and left there to rest for five minutes. This ensured that the fish was able to process the zooplankton it had ingested in the last minutes before the end of the feeding trial. Thereafter the fish was first anesthetized by placing it for five minutes in a solution containing 0.75g/l MS222 and 0.35g/l baking soda (as buffer) and then the fish was euthanized by placing it into another solution for 10 minutes containing 1.5g/l MS222 with 0.75g/l baking soda. To confirm that the fish was euthanized, we left it for another 10 minutes in fresh water and checked for any gill or fin movement and then severed the spine at the base of the head prior to dissection. Directly after dissecting and opening the stomach, we flushed the gills, the pharynx and the stomach with water to retrieve all caught zooplankton. We counted the total number of zooplankton under a binocular in a Bogorov Counting Chamber and distinguished between 1. all kinds of zooplankton with the majority belonging to *Copepods* and 2. *Daphnia*. The fish were labelled and preserved in 85% ethanol. We counted the number of zooplankton twice and used the mean from these two counts for further analysis. For lacerated zooplankton that was already separated into several pieces we counted the head only as one individual. To account for measurement error we calculated the percentage difference in number of counted zooplankton by dividing the difference between the two counts of zooplankton by the mean number of zooplankton from both counts. The mean percentage difference for the number of counted zooplankton was lower than 3%, indicating that measurement error was low. The mean difference in the number of zooplankton counted from the fish (N=50) was 3±3.5 (standard deviation). On the days where fish were tested for zooplankton we additionally preserved ten replicates of the same amount of zooplankton in ethanol to count the approximate number of zooplankton taxa offered per trial. We counted replicate samples under a binocular in a Bogorov Counting Chamber. Out of the fifty cells of the Chamber we randomly chose 10 cells and counted the number of zooplankton in each cell. The extrapolation of the average zooplankton count from those 10 cells approximated the total number of zooplankton of a sample. The mean number of zooplankton in the ten samples was 2794±225 and ranged between 2425 and 3137. This corresponds to a density of 112±9 zooplankton per liter. We distinguished between 1. all kinds of zooplankton with the majority

belonging to *Copepods* and 2. *Daphnia* and found that the mean percentage of *Daphnia* in a sample was 5.4±4.8 with a range of between 0.7 and 15.5 percent. This large variation in the percentage of *Daphnia* was not found in the zooplankton that was consumed by the fish. The mean percentage of *Daphnia* found in the fish (N=44; a total of fifty fish were tested on zooplankton, but for six fish no data existed on the percentage of *Daphnia*) was 1.1±2 and ranged between 0 and 8.9 percent. Based on a Kruskal-Wallis test the variation in the percentage of *Daphnia* in the three parental species and the two hybrid crosses did not differ (H=6.32, p=0.177).

Appendix 6: Differences in size, weight, trial length, latency and possible trial learning effects

Size and weight differences

Size and weight differences between the parental species and the hybrids were tested with a Kruskall-Wallis test. If significant, we additionally performed a pairwise Mann-Whitney U-test post-hoc. Not all individuals were tested on all food types, and thus we treated each food type as a separate dataset and tested for weight and size differences among the parental species and the hybrids tested in each of these datasets. In general, the parental species and the hybrids did not differ in size or weight when tested for each food type separately. The only exception to this was in the AC/PN/ACxPN dataset when tested on gammarids, where *A. calliptera* showed a trend towards being heavier and larger than *P. sp. 'nyererei-like'* (P=0.066 and P=0.056, respectively) (Table S3).

Trial length differences

The feeding trials on the algal substitute were generally terminated after forty minutes and on shrimps, gammarids and zooplankton after ten minutes. Due to practical constraints in some cases trials ran longer or shorter than planned. This happened in trials with all of the four food types. We tested if trial lengths differed between the parental species and the hybrids in both datasets separately with a Kruskal-Wallis test, and if significant, we additionally performed a pairwise Mann-Whitney U-test as post-hoc test. The trial length of the parental species and hybrids significantly differed for the PN/NO/PNxNO and AC/PN/ACxPN dataset tested on gammarids and for the AC/PN/ACxPN dataset tested on the algal substitute (Kruskall-Wallis test, Table S4). In the PN/NO/PNxNO dataset tested on gammarids the trial length of the hybrids was significantly longer than that of either parental species (Mann-Whitney U test, both P<0.001) (Table S4). In the AC/PN/ACxPN dataset tested on gammarids *A. calliptera* had significantly longer trials than the *P. sp. 'nyererei-like'* (P=0.006). When tested on the algal substitute the trial lengths of *A. calliptera* and of the hybrids were significantly longer when compared to that of *P. sp. 'nyererei-like'* (P=0.03 and P=0.017, respectively). Because of the significant difference in trial length in some cases, we used trial length as a fixed effect in multiple regression models on feeding efficiency (see below).

Latency differences

We measured latency as the time (in seconds) it took an individual to attack a prey item (e.g. zooplankton, gammarids or shrimps) or take a first bite of the algal substitute). *Latency* differences between the parental species and the hybrids were tested with a Kruskall-Wallis test. If significant, we additionally performed a pairwise Mann-Whitney U-test post-hoc. Not all individuals were tested on all food types, and thus we treated each food type as a separate dataset, and tested for latency differences among the parental species and the hybrids tested in each of these datasets. In two cases the generalist parental species *A. calliptera* showed a significantly lower latency than the first-generation hybrid cross ACxPN or the parental species *P. sp. 'nyererei-like'* in attacking a shrimp or taking a first bite of the algal substitute (AC/PN/ACxPN dataset tested on the algal substitute and on shrimp, all $P<0.03$, Table S7) and in one case the first-generation hybrid cross PNxNO showed a significantly lower latency than then parental species *N. omnicaerleus* in attacking a shrimp (PN/NO/PNxNO dataset tested on shrimp, $P=0.005$, Table S7).

Trial learning effect

Individual fish were tested multiple times on three of the four food types (gammarids, shrimps, algal substitute). We used all individuals that were successfully tested in three feeding trials to test for a possible learning effect. We tested with a repeated measures ANOVA if individuals in each parental species and hybrid cross or all individuals showed a learning effect on different food types, i.e. if their feeding efficiency improved or decreased from the first to the second and third feeding trial. No learning effect was observed on any of the four food types, neither for individuals of the parental species nor the hybrids, nor when all individuals were analysed together (Table S8).

Table S1

Given is the number of fish of each parental species and their hybrids that were tested in each experimental batch, the order among food type trials in which the fish of each experimental batch were tested and in brackets the total number of trials.

		Number of fish of parental species or F1 hybrid cross					Trial order for the tested food types (total number of trials per group)			
Experimental batch	**Number of fish tested**	**PN**	**NO**	**AC**	**PNxNO**	**ACxPN**	**First**	**Second**	**Third**	**Forth**
1	4	3	1				Algal substitute (13)	Shrimps (10)	Gammarids (12)	Zooplankton (4)
2	4	1	3				Shrimps (13)	Algal substitute (12)	Gammarids (12)	Zooplankton (4)
3	12	4	4		4		Gammarids (35)	Shrimps (29)	Algal substitute (24)	Zooplankton (11)
4	16	2	2	4	4	4	Gammarids (46)	Shrimps (50)	Algal substitute (38)	Zooplankton (15)
5	16	1		5	4	6	Shrimps (40)	Gammarids (30)	Algal substitute (31)	Zooplankton (15)
Total number of fish	**52**	**11**	**10**	**9**	**12**	**10**				

Table S2.

Given is the number of feeding trials per food type (Algal substitute, gammarid, shrimp) that could be used in the linear mixed models to calculate the feeding performance. In the majority of cases three trials per individual were obtained and only occasionally would there be less or more trials on one food type (from 1 to 5).

	Total number of fish	**Number of trials per individual on algal substitute**				
Species or F1 hybrid cross		**1**	**2**	**3**	**4**	**5**
PN	**9**		2	6	1	
NO	**9**		1	7	1	
AC	**6**		1	5		
PNxNO	**9**	1	1	5	2	
ACxPN	**7**	1		4	1	1
	Total number of fish	**Number of trials per individual on gammarids**				
Species or F1 hybrid cross		**1**	**2**	**3**	**4**	**5**
PN	**10**			10		
NO	**10**			9	1	
AC	**7**		1	4	2	
PNxNO	**11**	1		10		
ACxPN	**7**			7		
	Total number of fish	**Number of trials per individual on shrimps**				
Species or F1 hybrid cross		**1**	**2**	**3**	**4**	**5**
PN	**9**	1	1	6	1	
NO	**10**			6	4	
AC	**7**			6	1	
PNxNO	**10**		1	5	4	
ACxPN	**9**		1	7	1	

Table S3

The differences in weight and size between the parental species and hybrids from the two datasets were tested on each food type separately (PN/NO/PNxNO and AC/PN/ACxPN) with a Kruskall-Wallis test and a Mann-Whitney U test as post hoc test. P values from the Mann-Whitney U test are adjusted for multiple comparisons with a false discovery rate of 0.05 (Benjamini and Hochberg, 1995; Verhoeven et al. 2005). Significant P-values (<0.05) are in bold.

Weight	Food type	Dataset	Kruskal-Wallis test	PN vs. NO or AC	PN vs. F1 hybrid	NO or AC vs. F1 hybrid
	Algal subsitute	PN/NO/PNxNO	H=0.24, p=0.887			
		AC/PN/ACxPN	H=4.82, p=0.090			
	Gammarids	PN/NO/PNxNO	H=1.22, p=0.543			
		AC/PN/ACxPN	**H=6.33, p=0.042**	0.066	0.283	0.187
	Shrimps	PN/NO/PNxNO	H=0.46, p=0.800			
		AC/PN/ACxPN	H=3.12, p=0.210			
	Zooplankton	PN/NO/PNxNO	H=031, p=0.858			
		AC/PN/ACxPN	H=3.84, p=0.146			

Size	Food type	Dataset	Kruskal-Wallis test	PN vs. NO or AC	PN vs. F1 hybrid	NO or AC vs. F1 hybrid
	Algal subsitute	PN/NO/PNxNO	H=0.77, p=0.682			
		AC/PN/ACxPN	H=5.94, p=0.052			
	Gammarids	PN/NO/PNxNO	H=2.81, p=0.245			
		AC/PN/ACxPN	**H=6.86, p=0.027**	0.056	0.105	0.125
	Shrimps	PN/NO/PNxNO	H=0.69, p=0.709			
		AC/PN/ACxPN	H=3.66, p=0.160			
	Zooplankton	PN/NO/PNxNO	H=0.85, p=0.652			
		AC/PN/ACxPN	H=4.03, p=0.134			

Table S4

Differences in trial lengths between the parental species and hybrids from the two datasets were tested on each food type separately (PN/NO/PNxNO and AC/PN/ACxPN) with a Kruskall-Wallis test and a Mann-Whitney U test as post hoc test. P values from the Mann-Whitney U test are adjusted for multiple comparisons with a false discovery rate of 0.05 (Benjamini and Hochberg, 1995; Verhoeven et al. 2005). Significant P-values (<0.05) are in bold.

Food type	Dataset	Kruskal-Wallis test	PN vs. NO or AC	PN vs. F1 hybrid	NO or AC vs. F1 hybrid
Algal subsitute	PN/NO/PNxNO	H=2.13, P=0.345			
	AC/PN/ACxPN	**H=9.59, P=0.008**	**0.03** **PN < AC**	**0.017** **PN < F1**	0.479
Gammarids	PN/NO/PNxNO	**H=24.90, P<0.001**	0.22	**<0.001** **PN < F1**	**<0.001** **AC < F1**
	AC/PN/ACxPN	**H=8.43, P=0.015**	**0.006** **PN < AC**	0.534	0.189
Shrimps	PN/NO/PNxNO	H=2.33, P=0.312			
	AC/PN/ACxPN	H=4.26, P=0.119			
Zooplankton	PN/NO/PNxNO	H=2.29, P=0.318			
	AC/PN/ACxPN	H=5.56, P=0.062			

Table S5

ANOVA results from linear mixed models with feeding efficiency as response variable (in all models log_{10} transformed). Separate linear mixed models on the feeding efficiency on the four food types were run for each dataset (PN/NO/PNxNO and AC/PN/ACxPN). The feeding efficiency model included the fixed effects "Group" (the three parental species and both first-generation hybrid crosses are each one group), "BS" (=body size measured as standard length of the fish) and "Trial length" (the duration of an experimental trial). Random factors in the model were "Individual" (used in the models for the food types where individual fish were tested repeatedly: algal substitute, gammarid and shrimp) and "Experimental batch" (used in all models). The sum of squares (SS), degrees of freedom numerator (DFN) and denominator (Satterthwaite approximated and rounded to an integer, DFD), F- and P-values (F, P) for the different sets of fixed effects used in the models are given. For assessing the fit of each model the root mean square error (RSME) between the predicted and observed values and the AIC were calculated. We calculated the relative-impact value (r-i value) of the three fixed factors by applying additive models including all combinations of each of the three factors ("Group", "BS", "Trial length"). Significant P-values (<0.05) for a given fixed effect or an interaction between two fixed effects are in bold.

Food type	Dataset	AIC	RSME		Group*BS	Group	BS	Trial Length
				DFN	2	2	1	1
Algal substitute	PN/NO/PNxNO	115.9	0.3	SS, DFD	0.03, 20	0.013, 20	0.14, 18	0.07, 70
				F	0.13	0.06	1.24	0.68
				P	0.88	0.94	0.281	0.41
				r-i value		0.98	0.04	<0.001
	AC/PN/ACxPN	78.5	0.23	SS, DFD	0.33, 15	0.33, 15	0.44, 15	0.14, 57
				F	2.44	2.45	6.73	2.1
				P	0.12	0.12	**0.02**	0.15
				r-i value		0.63	0.41	0
Gammarids	PN/NO/PNxNO	18.87	0.17	SS, DFD	0.18, 23	0.15, 23	0.35, 22	0.03, 75
				F	2.82	2.39	11.2	0.84
				P	0.08	0.114	**0.003**	0.36
				r-i value		0.43	0.78	<0.001
	AC/PN/ACxPN	39.32	0.17	SS, DFD	0.04, 16	0.20, 16	0.08, 16	0.02, 64
				F	0.51	0.49	2.22	0.54
				P	0.61	0.624	0.156	0.47
				r-i value		0.63	0.38	0
Shrimps	PN/NO/PNxNO	46.93	0.21	SS, DFD	0.10, 48	0.11, 51	0.01, 79	<0.01, 79
				F	1.09	1.13	0.1	0.16
				P	0.34	0.329	0.744	0.69
				r-i value		1	0.01	<0.001
	AC/PN/ACxPN	139	0.34	SS, DFD	0.31, 18	0.26, 18	<0.001, 18	0.23, 66
				F	1.07	0.87	0.003	1.56
				p	0.36	0.433	0.957	0.22
				r-i value		0.94	0.06	<0.01
Zooplankton	PN/NO/PNxNO	43.8	0.17	SS, DFD	0.18, 11	0.12, 11	<0.001, 18	0.03, 14
				F	2.38	1.62	<0.001	0.8
				P	0.14	0.247	0.99	0.39
				r-i value		1	0.01	<0.001
	AC/PN/ACxPN	36.04	0.13	SS, DFD	0.29, 19	0.31, 19	<0.001, 20	0.002, 20
				F	6.02	6.47	0.01	0.1
				P	**0.01**	**0.007**	0.922	0.76
				r-i value		0.99	0.02	<0.001

Table S6

Differences in feeding efficiency between groups calculated from the least-squares means and confidence intervals of each group. The least-square means and CI were extracted from the linear mixed feeding efficiency model (Table S5) for each food type and dataset (PN/NO/PNxNO and AC/PN/ACxPN) separately. Shown are the pair-wise means (positive mean indicate that the first group has a higher feeding efficiency compared to the second group), the standard error (SE), the degrees of freedom (DF), the test statistics (T- and raw P-value) and the false discovery rate (FDR) adjusted P-values (P_{FDR}). Significant P-values (raw and adjusted) and their corresponding means are in bold.

Food type	Dataset	Pair-wise differences	Mean	SE	DF	T	P	P_{FDR}
Algal substitute	PN/NO/PNxNO	NO vs. PN	**0.34**	0.11	18.3	3.1	**0.006**	**0.018**
		PN vs. PNxNO	-0.19	0.122	20.3	-1.58	0.129	0.194
		NO vs. PNxNO	0.15	0.114	17.4	1.31	0.206	0.206
	AC/PN/ACxPN	AC vs. PN	-0.02	0.13	17.4	-0.12	0.91	0.91
		ACxPN vs. PN	**0.29**	0.123	16	2.35	**0.03**	**0.045**
		AC vs. ACxPN	**-0.3**	0.129	14.3	-2.35	**0.03**	**0.045**
Gammarids	PN/NO/PNxNO	NO vs. PN	**0.17**	0.053	21.4	3.16	**0.005**	**0.008**
		PN vs. PNxNO	**-0.23**	0.061	26	-3.72	**0.001**	**0.003**
		NO vs. PNxNO	-0.06	0.058	26.2	-1.04	0.308	0.308
	AC/PN/ACxPN	AC vs. PN	0.16	0.099	18.6	1.67	0.1	0.3
		ACxPN vs. PN	0.07	0.093	18.2	1.06	0.3	0.4
		AC vs. ACxPN	0.1	0.077	16.8	0.86	0.4	0.4
Shrimps	PN/NO/PNxNO	NO vs. PN	**0.12**	0.06	82.1	2.08	**0.04**	**0.04**
		PN vs. PNxNO	**-0.27**	0.061	61.8	-4.42	**<0.001**	**<0.001**
		NO vs. PNxNO	**-0.15**	0.056	52.3	-2.63	**0.01**	**0.015**
	AC/PN/ACxPN	AC vs. PN	**0.42**	0.201	15.5	2.1	**0.05**	0.09
		ACxPN vs. PN	0.4	0.19	13.8	2.08	0.06	0.09
		AC vs. ACxPN	0.03	0.164	15.5	0.17	0.87	0.87
Zooplankton	PN/NO/PNxNO	NO vs. PN	**-0.44**	0.09	23.8	-4.9	**<0.001**	**<0.001**
		PN vs. PNxNO	0.02	0.091	19.9	-0.17	0.9	0.9
		NO vs. PNxNO	**-0.42**	0.089	21.9	-4.74	**<0.001**	**<0.001**
	AC/PN/ACxPN	AC vs. PN	**-0.42**	0.088	21	-4.81	**<0.001**	**<0.001**
		ACxPN vs. PN	**-0.19**	0.088	21	-2.15	**0.043**	**0.043**
		AC vs. ACxPN	**-0.23**	0.079	18.1	-2.93	**0.009**	**0.014**

Table S7

Differences in latency between the parental species and hybrids from the two datasets were tested on each food type separately (PN/NO/PNxNO and AC/PN/ACxPN) with a Kruskall-Wallis test and a Mann-Whitney U test as post hoc test. P values from the Mann-Whitney U test are adjusted for multiple comparisons with a false discovery rate of 0.05 (Benjamini and Hochberg, 1995; Verhoeven et al. 2005). Significant P-values (<0.05) are in bold.

Food type	Dataset	Kruskal-Wallis test	PN vs. NO or AC	PN vs. F1 hybrid	NO or AC vs. F1 hybrid
Algal subsitute	PN/NO/PNxNO	H=2.13, P=0.345			
	AC/PN/ACxPN	**H=9.59, P=0.008**	**0.03 PN<AC**	0.479	**0.017 AC>F1**
Gammarids	PN/NO/PNxNO	H=2.33, P=0.311			
	AC/PN/ACxPN	H=1.61, P=0.448			
Shrimps	PN/NO/PNxNO	**H=10.18, P=0.006**	0.246	0.113	**0.005 NO<F1**
	AC/PN/ACxPN	**H=10.38, P=0.006**	**0.013 PN<AC**	0.256	**0.013 AC>F1**
Zooplankton	PN/NO/PNxNO	H=1.78, P=0.411			
	AC/PN/ACxPN	H=0.01, P=0.996			

Table S8

Test statistics from a repeated measures ANOVA testing if individuals in each of the parental species or hybrid crosses or if in general all individuals (termed "All") showed a learning effect on each food type, i.e. if the feeding efficiency improved or decreased throughout the three feeding trials.

Repeated measures ANOVA

Food type	Parental species or F1 hybrid cross	F statistics and P-value
Algal substitute	PN	F(2,12)=1.04, P=0.385
	NO	F(2,14)=0.39, P=0.685
	AC	F(2,8)=1.20, P=0.349
	PNxNO	F(2,12)=0.91, P=0.430
	ACxPN	F(2,10)=1.26, P=0.324
	All	F(1,4)=1.65, P=0.268
Gammarids	PN	F(2,18)=0.61, P=0.553
	NO	F(2,18)=1.37, P=0.280
	AC	F(2,10)=1.11, P=0.369
	PNxNO	F(2,18)=1.70, P=0.211
	ACxPN	F(2,12)=1.19, P=0.339
	All	F(1,4)=2.17, P=0.215
Shrimps	PN	F(2,12)=0.39, P=0.684
	NO	F(2,18)=0.26, P=0.773
	AC	F(2,12)=0.15, P=0.860
	PNxNO	F(2,16)=1.25, P=0.314
	ACxPN	F(2,16)=1.85, P=0.194
	All	F(1,4)=0.13, P=0.738

References

Barel, C. D.N. 1983. Toward a constructional morphology of cichlid fishes (Teleostei, Perciformes). Netherlands J. Zool. 33:357–424.

Bouton, N., De Visser, J. & Barel, C. D. N. 2002. Correlating head shape with ecological variables in rock-dwelling haplochromines (Teleostei : Cichlidae) from Lake Victoria.

Biol. J. Linnean Soc. 76: 39-48.

Bouton, N., Seehausen, O. and van Alphen, J. J. M. 1997. Resource partitioning among rock dwelling haplochromines (Pisces : Cichlidae) from Lake Victoria. Ecol. Fresh. Fish 6: 225-240.

Bouton, N., van os, N. & Witte, F. 1998. Feeding performance of Lake Victoria rock cichlids: testing predictions from morphology. J. Fish Biol.

Bouton, N., Witte, F., van Alphen, J.J.M., Schenk, A. & Seehausen, O. 1999. Local Adaptations in Populations of Rock-Dwelling Haplochromines (Pisces: Cichlidae) from Southern Lake Victoria. Proc. R. Soc. Lond. B 266:355-360.

Bouvier, E.-L., 1904. Crevettes de la famille des Atyidés: espèces qui font partie des collections du Muséum d'Histoire naturelle. Bull. Mus. Hist. nat. Paris. 10:129-138.

Greenwood, P.H. 1979. Towards a phyletic classification of the 'genus' Haplochromis (Pisces, Cichlidae) and related taxa. Part 1. Bull. Brit. Mus. (Nat. Hist.) Zool. 35: 265-322.

Goudswaard, K. P. C., Witte, F. & Wanink, J. H. 2006. The shrimp Caridina nilotica in Lake Victoria (East africa), before and after the Nile perch increase. Hydrobiologia 563: 31-44.

Günther, A. 1894. Second report on the reptiles, batrachians, and fishes transmitted by Mr.H. H. Johnston, C. B., from British Central Africa. Proceedings of the General Meetings for Scientific Business of the Zoological Society of London 4:616–628.

Hulsey, C.D. & García de León, F.J. 2005. Cichlid jaw mechanics: linking morphology to feeding specialization. Funct. Ecol. 19:487-494.

Meier JI, Sousa VC, Marques DA, Selz OM, Wagner CE, Excoffier L, Seehausen O. 2017 Demographic modelling with whole-genome data reveals parallel origin of similar *Pundamilia* cichlid species after hybridization. *Mol. Ecol.* 26, 123-141.

McGee, M.D and Wainwright, P.C. 2013. Sexual dimorphism in the feeding mechanism of threespine stickleback. J. Exp. Biol. 216:835-840.

Mwebaza-Ndawula, L. 1994. Changes in relative abundance of zooplankton in northern Lake Victoria, East Africa. Hydrobiologia 272:259–264.

Parsons, K. J., Taylor, A. T., Powder, K. E. & Albertson, R. C. 2014. Wnt signaling underliesthe evolution of new phenotypes and craniofacial variability in Lake Malawi cichlids. Nature Communications. DOI:10.1038/ncomms4629.

Roux, P. 1833. Lettre relative a divers Coquilles, Crustacés. Insectes, Reptiles et Oiseaux, observés en Égypte. Annales des Sciences Naturelles Paris 28:72–78.

Rupp, M.F. & Hulsey, C.D. 2014. Influence of substrate orientation on feeding kinematics and performance of algae grazing Lake Malawi cichlid fishes. J. Exp. Biol. DOI:10.1242/jeb.105080.

Seehausen, O., Lippitsch, E., Bouton, N. & Zwennes, H. 1998. Mbipi, the rock-dwelling cichlids of Lake Victoria: description of three new genera and fifteen new species (Teleostei). Ichthyol.

Explor. Freshw. 9:129–228.

Seehausen, O. & Bouton, N. 1998. The community of rock-dwelling cichlids in Lake Victoria. Bonn. zool. Beitr. 47:3-4.

Stelkens, R. B., Schmid, C., Selz, O. & Seehausen, O. 2009. Phenotypic novelty in experimental hybrids is predicted by the genetic distance between species of cichlid fish. BMC Evol. Biol. 9:283.

Tkint, T., Verheyen, E., De Kegel, B., Helsen, P. & Adriaens, D. 2012. Dealing with Food and Eggs in Mouthbrooding Cichlids: Structural and Functional Trade-Offs in Fitness Related Traits. PloS One 7:e31117.

Wainwright, P.C., Ferry-Graham, L.A., Waltzek, T.B., Carroll, A.M., Hulsey, C.D. & Grubich, J.R. 2001. Evaluating the use of ram and suction during prey capture by cichlid fishes. J. Exp. Biol. 204: 3039–3051.

Witte-Maas, E.L.M. & Witte, F. 1985. Haplochromis nyererei, a new cichlid fish from Lake Victoria named in honour of Mwalimu Julius Nyerere, President of Tanzania. Leiden (The Netherlands): Brill.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Lond. B, 57: 289–300.

Verhoeven, K.J.F, Simonsen, K.L. & McIntyre, L.M. 2005. Implementing false discovery rate control: increasing your power. Oikos 108:643–647.