Modular and redundant genomic architecture underlies combinatorial mechanism of speciation and adaptive radiation

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Abstract

Hybridisation can fuel rapid adaptive radiation, but how it enables the formation of phenotypically highly dimensional species-rich radiations remains unclear. We investigated this by analysing genotype-phenotype associations for 14 ecological (trophic, body patterns) and mating traits (nuptial colour) across 107 species of Lake Victoria cichlid fishes. We find weak trait covariance across the radiation, with many different trait combinations constituting different species. Across the radiation, polygenic, redundant, and lowly pleiotropic genomic architectures of hybrid origin underlie the repeated evolution of key traits. Such independent

genomic modules can be reshuffled and recombined like Lego bricks, generating diverse trait combinations from a finite number of elements. During speciation, dispersed oligogenic trait modules become coupled through long-range linkage disequilibrium. We propose that this genomic and phenotypic modularity emerged from repeated cycles of past hybridisation, enabling superfast adaptive radiation through combinatorial speciation.

Key words: adaptive radiation, speciation, genetic architecture, pleiotropy, genetic redundancy, hybridisation

Main Text:

Adaptive radiation, where an ancestral lineage diversifies quickly into many eco-morphologically diverse species that can also quickly coexist in species rich communities (non-geographical radiation), may have been an important source of biodiversity in key moments of the 3.5-billion-year history of Life on Earth (*1–3*). Its occurrence is highly punctuated in space, time, and phylogeny, and despite its likely importance, knowledge of the biological factors that trigger, facilitate, and constrain this process is incomplete (*4*, *5*). It is widely accepted that exceptional ecological opportunity is a prerequisite (*6*), but only some of many possible lineages seem to ever have responded to exceptional ecological opportunity with adaptive radiations and even fewer have done so rapidly and repeatedly. From studying some of the geologically youngest radiations, it is apparent that the process can sometimes unfold in just a few thousand years (*7–9*).This is consistent with the fossil evidence for long periods of relative stasis, punctuated by bursts of diversification in the history of life(*10*, *11*). Such species radiations, with many speciation events in succession are far too fast for intrinsic reproductive isolation to have emerged before species assemble into rich sympatric communities. But they are also far too young for adaptive variation to have arisen through mutations and tend to be characterised by the coincidence of hybrid ancestry and/or recurrent hybridization and simultaneous evolution of ecological and mating traits, as was predicted by Anderson & Stebbins 70 years ago (*10*). While some inroads have been made linking rapid evolution to the availability of ancient admixture variation in plants and animals (*9*, *12–17*), we still understand little about the genetic basis that not only allows key traits to evolve rapidly, but that permits the rapid generation of many different highly dimensional phenotypes that are associated with many speciation events in short succession in the absence of geographical isolation.

Research has illuminated two prevalent genetic architectures underlying ecological speciation without geographical isolation. These architectures are either (1) simple and consist of a few genomic 'islands' of differentiation made up of either clustered small effect alleles physically linked into a supergene or single loci of large effect (*18–21*) or (2) more complex and involve genome-wide and/or polygenic patterns of

differentiation (*22–33*). Each of these architectures imposes unique constraints on speciation in the face of gene flow (*23*, *34*). Under the scenario of simple concentrated trait architectures, phenotypic differentiation may be maintained despite some gene flow because total selection will be distributed over one or a few regions in the genome and may hence be strong enough to counter gene flow at those loci and also because large effects of single loci cannot be broken down by recombination (*35*, *36*). However, speciation is improbable in the first place unless if the locus under divergent selection also causes reproductive isolation as a by-product, e.g. in the form of 'magic traits' (*37–39*) or through physical linkage to loci causing reproductive isolation (*40–42*). Indeed, adaptive polymorphism within species have been attributed to such genetic architectures and have proven not to be associated with speciation, including in cichlid fish (*43*, *44*). When trait architectures are complex such that expression of divergent trait values requires many small to medium effect alleles distributed over multiple chromosomes, it is more likely that some of these loci are either physically linked to genes affecting mating compatibility or affect the latter through pleiotropy. If not, the strength of divergent selection per locus will likely be insufficient to counteract the homogenising effects of gene flow at any one locus, making speciation without geographic isolation unlikely (*34*, *45*). Theory suggests that the latter constraint can be overcome either through the gradual build-up of weak polygenic Linkage Disequilibrium (LD) at many loci across the genome over millions of years, followed by rapid transition to two species around genomic 'tipping points'(*46*) or through hybridisation introducing many divergent haplotypes with multiple linked SNPs at once (*47*, *48*). Neither of these theories have robustly been tested empirically in any adaptive radiation. Furthermore, most theoretical speciation-with-gene flow models were built to explain singular speciation events where the processes of accumulation of variation and building of LD restart after every speciation event. Such models cannot easily be applied to adaptive radiations with tens or hundreds of speciation events that occurred within ten or hundred thousand years (but see (*47*, *49*)).

Adaptive radiations that produce many sympatric species, whether in cichlids, Darwin's Finches or Hawaiian Honeycreepers, are associated with high dimensionality of phenotypic and ecological variation even early on in the process. Hence, selection was most likely diverse and multifarious. Few theoretical and even fewer empirical studies have considered the genetic architecture that allows many successive or simultaneous speciation events when the interaction of multiple environmental factors impose divergent selection pressure on multiple traits and trait combinations, and when there is not sufficient time between successive speciation events for recovery of genetic variation through mutation (*48*, *50*). Here, we addressed this problem in the fastest and most species-rich cichlid fish adaptive radiation known by conducting radiation-wide genotype-phenotype association analyses of hundreds of individuals that represent 107 species and 14 ecological and mate-choice traits (Fig. 1).

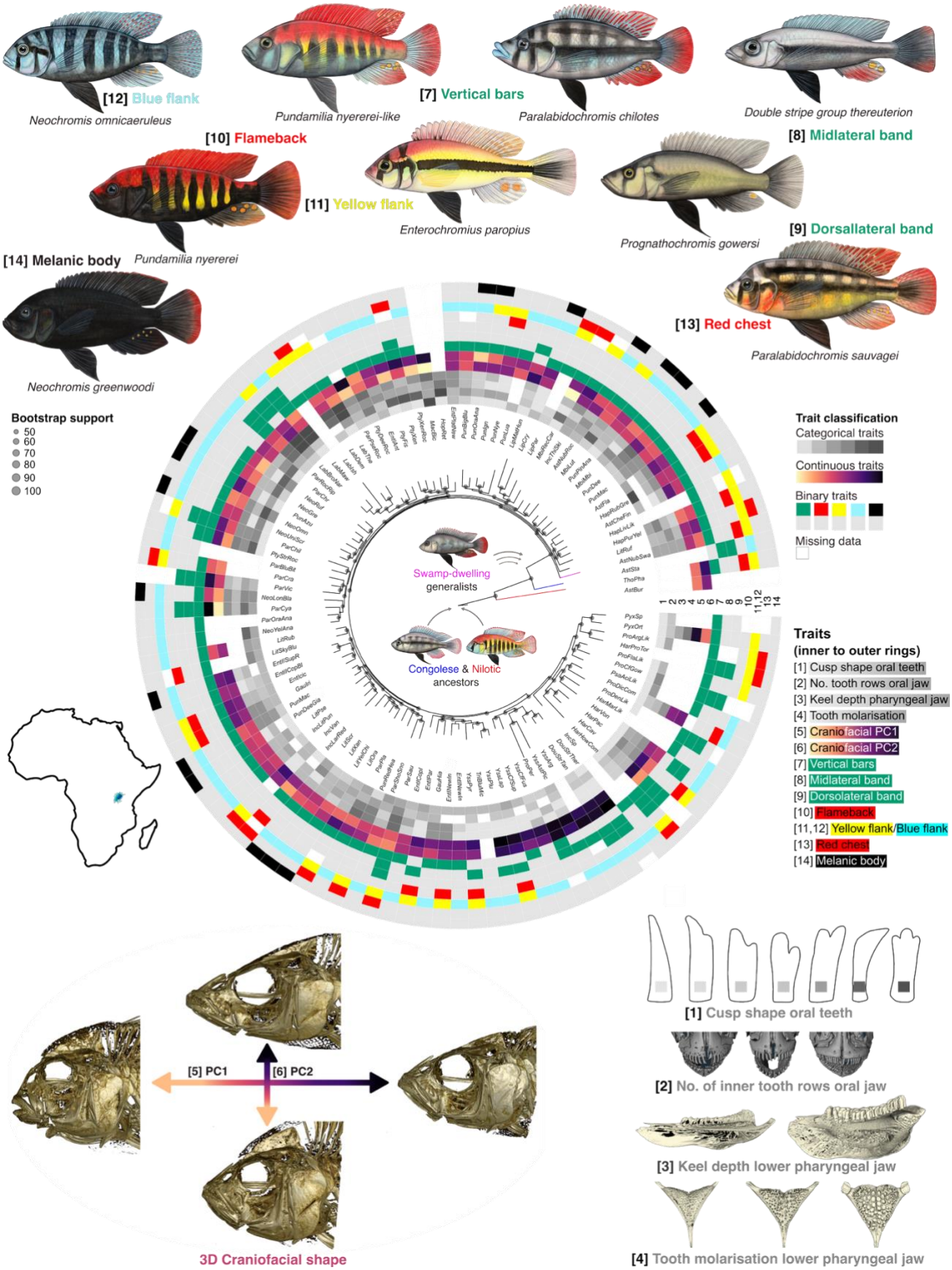

[12] Blue flank
Neochromis omnicaeruleus
Pundamilia nyererei-like
[7] Vertical bars
Paralabidochromis chilotes
Double stripe group thereuterion
[10] Flameback
[8] Midlateral band
[11] Yellow flank
Enterochromius paropius
Prognathochromis gowersi
[9] Dorsallateral band
[14] Melanic body
Pundamilia nyererei
[13] Red chest
Paralabidochromis sauvagei
Neochromis greenwoodi
Bootstrap support
50
60
70
80
90
100
Trait classification
Categorical traits
Continuous traits
Binary traits
Missing data
Swamp-dwelling generalists
Congolese & Nilotic ancestors
Traits
(inner to outer rings)
[1] Cusp shape oral teeth
[2] No. tooth rows oral jaw
[3] Keel depth pharyngeal jaw
[4] Tooth molarisation
[5] Craniofacial PC1
[6] Craniofacial PC2
[7] Vertical bars
[8] Midlateral band
[9] Dorsolateral band
[10] Flameback
[11,12] Yellow flank/Blue flank
[13] Red chest
[14] Melanic body
[5] PC1
[6] PC2
3D Craniofacial shape
[1] Cusp shape oral teeth
[2] No. of inner tooth rows oral jaw
[3] Keel depth lower pharyngeal jaw
[4] Tooth molarisation lower pharyngeal jaw

Fig. 1 Overview of ecological and mating trait evolution in the Lake Victoria cichlid adaptive radiation. The Lake Victoria cichlid radiation encompasses ~500 known species (107 mostly sympatric species shown in the phylogeny) that represent unique phenotypic and genetic mosaics of recurrent ecomorphologies, melanic stripe patterns, and male nuptial colour motifs (illustrated with species shown), assembled in <16,000 years through combinatorial mechanisms (*51*). Distribution of 14 core traits used in this study are shown in 13 rows (traits 11 and 12 are mutually exclusive traits and thus plotted in the same row)around the genome-wide SNP phylogeny (constructed using *P. nyererei* v3 reference genome here) in such a way that each column ray around the circle tree represents the unique set of trait combinations present in the species at the corresponding tip of the tree. Ecomorphological traits are numbered from 1 – 6: cusp shape of outer row frontal teeth [1] and number of inner tooth rows in oral jaws [2], lower pharyngeal jaw keel depth [3] and molarisation of lower pharyngeal jaw teeth [4], as well are 3-dimensional (3D) craniofacial shape PC1 [5] and PC2 [6]. Colour gradients of PC1 and PC2 arrows correspond to the PC value heatmap as illustrated around the phylogeny. Melanic stripe patterning traits are numbered from 7 – 9: vertical bars [7], midlateral band [8] and dorsolateral band [9], where the latter only occurs in combination with [8]. Male nuptial colour motifs are numbered from 10 – 13: flameback [10], yellow or blue flank [11,12], red chest [13], and melanic body [14]. Traits 1 – 4 are categorical (see Fig. S1 for all categories), traits 5 and 6 are continuous, and traits 7 – 14 are coded as binary (presence/absence). Node bootstrap support as circles of increasing sizes. Key ancestral admixture events in the ancestry of the radiation from (*9*) are shown. Distribution of ecological and mating traits across the phylogeny suggests that trait modules can largely recombine freely into different combinations in species.

Low covariance among phenotypic modules of adaptive radiation

If admixture variation from the hybrid swarm origin of the radiation was important to permitting exceptionally fast onset of speciation and adaptive radiation when the new lake formed, and if continued syngameon conditions in the emerging radiation were key for maintaining the momentum through many successive speciation events, we expect a radiation-wide genome panel to uncover the recycled component of the genetic architecture of key traits associated with repeated use of the same alleles for the same traits across the radiation, albeit in different trait combinations in different species (*52*). We explored this expectation with a Radiation-Wide genotype-phenotype Association Study (RWAS) after generating a long-read improved reference genome assembly and Iso-seq based gene annotation (Table S1). To this end we conducted the most comprehensive phenotyping in any cichlid adaptive radiation to date and analysed genomes of 324 individuals

from 107 different species (File S1). Unlike GWAS on a few species or a hybrid population, the RWAS approach is not designed to capture genetic variants that contribute to a trait in just one or two species but is designed to capture variants only if they affect the same trait in several species, which would be a prediction of the combinatorial hypothesis for adaptive radiation (*51*). We focused on 14 key traits involved in ecological adaptation and mating behaviour (Fig. 1) that make up three complementary trait complexes: (1) ecomorphology - we quantified craniofacial shape using 3D geometric morphometric from high resolution CT scans and summarised it as continuous variation along major axes of a Principal Component Analysis based on 3D geometric morphometrics from high resolution CT scans (Fig. S1); we categorised shape and arrangement of teeth in the oral jaws, as well as pharyngeal jaw shape and tooth molarisation into five to seven categories (Fig. S2); (2) melanic stripe patterns - presence/absence of vertical bars on the flank, a midlateral band, and a dorsolateral band (only occurs in conjunction with midlateral band) (*53*); (3) male nuptial colour motifs - we scored presence/absence of all known recurrent Lake Victoria cichlid male nuptial colour motifs (*54*, *55*): blue flank versus yellow flank, flameback (red-dorsum), red chest (red-ventrum), and melanic body (Fig. 1). Craniofacial shape, pharyngeal and oral jaw shape and dentition are all correlated with trophic ecology (*56*, *57*), melanic stripe patterns are thought to play a role in adaptation to different habitats and shoaling versus territorial behaviour (*53*) and male nuptial colouration is of critical importance in female mate choice, male territoriality and thus, species persistence in sympatry (*58–61*). Together, these 14 traits capture major aspects of the interspecific variation that characterises the Lake Victoria cichlid radiation (*56*, *57*).

Our phenotyping revealed that across the radiation these 14 traits may be quite freely recombined with each other, allowing a very large number of unique species phenotypes to be generated just from this trait set (see Fig. 1). A few pairs of correlated traits exist, but most traits appear to evolve independently of each other (mean absolute Spearman's ρ correlation coefficient 0.27; Fig. 2a). Noteworthy positive correlations exist between: yellow flank and flameback, whereas blue flank is exclusive of yellow flank and negatively correlated (but not exclusive) with flameback; midlateral and dorsolateral band, where the latter does not occur without the former; lower pharyngeal jaw keel depth and the extend of tooth molarisation. Noteworthy negative correlations exist between craniofacial shape PC1 (elongation of head and snout and upwards/downwards orientation of mouth) and number of tooth rows in the oral jaws (a high number of tooth rows is seen only in fish with downwards opening mouths); and between midlateral band and vertical bars. All of these recover well-known between-species trait associations (*56*, *62*, *63*). The fact that most other traits are only mildly correlated across the radiation is noteworthy because highly correlated traits would constrain

diversification to a limited number of unique phenotypes and species, while uncorrelated traits can be freely combined into many unique species in a modular fashion (*64*).

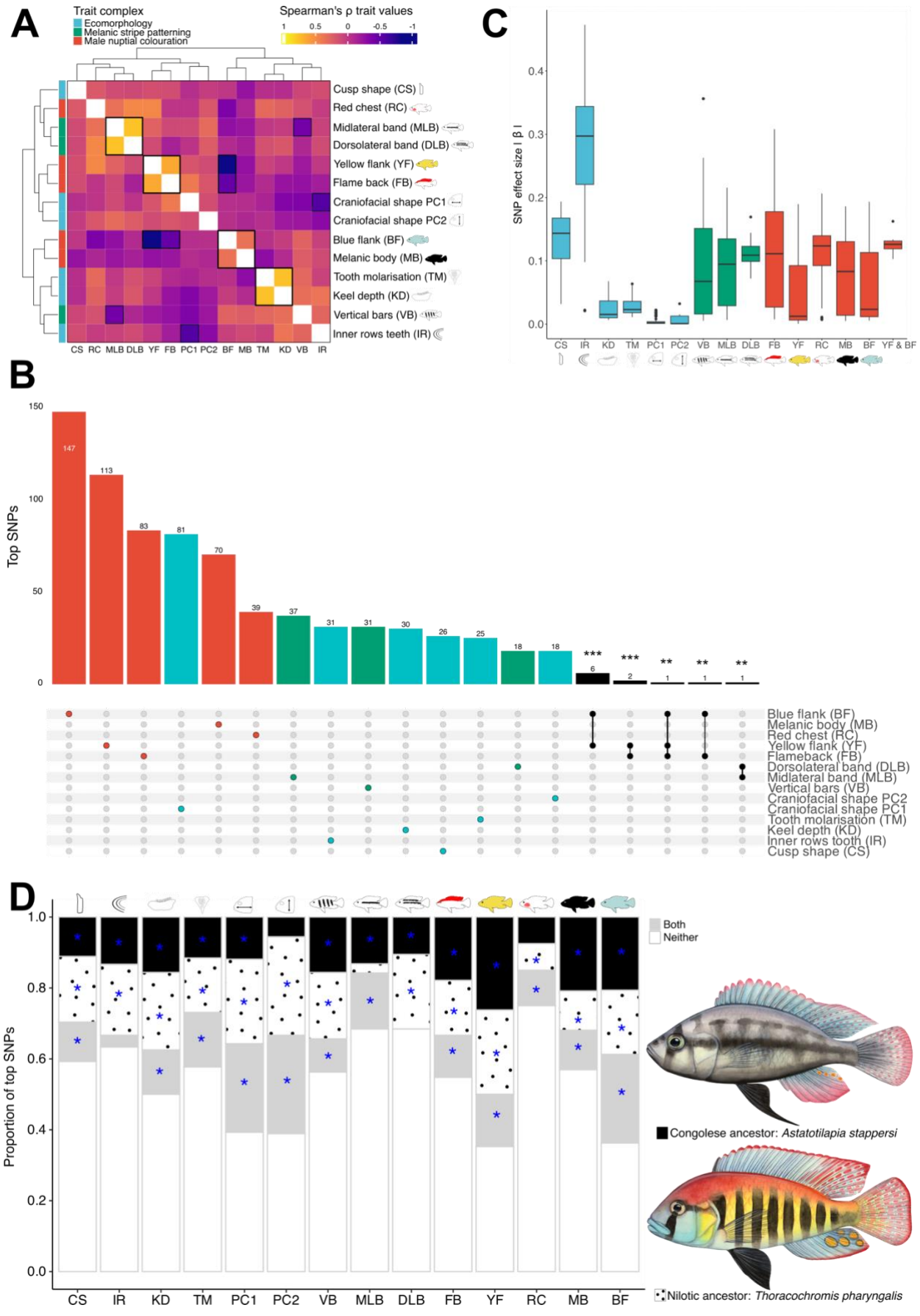

A
Trait complex
Ecomorphology
Melanic stripe patterning
Male nuptial colouration
Spearman's ρ trait values
1 0.5 0 -0.5 -1
Cusp shape (CS)
Red chest (RC)
Midlateral band (MLB)
Dorsolateral band (DLB)
Yellow flank (YF)
Flame back (FB)
Craniofacial shape PC1
Craniofacial shape PC2
Blue flank (BF)
Melanic body (MB)
Tooth molarisation (TM)
Keel depth (KD)
Vertical bars (VB)
Inner rows teeth (IR)
CS RC MLB DLB YF FB PC1 PC2 BF MB TM KD VB IR
C
SNP effect size | β |
CS IR KD TM PC1 PC2 VB MLB DLB FB YF RC MB BF YF & BF
B
Top SNPs
147 113 83 81 70 39 37 31 31 30 26 25 18 18 6 2 1 1 1
Blue flank (BF)
Melanic body (MB)
Red chest (RC)
Yellow flank (YF)
Flameback (FB)
Dorsolateral band (DLB)
Midlateral band (MLB)
Vertical bars (VB)
Craniofacial shape PC2
Craniofacial shape PC1
Tooth molarisation (TM)
Keel depth (KD)
Inner rows tooth (IR)
Cusp shape (CS)
D
Proportion of top SNPs
Both
Neither
Congolese ancestor: Astatotilapia stappersi
Nilotic ancestor: Thoracochromis pharyngalis
CS IR KD TM PC1 PC2 VB MLB DLB FB YF RC MB BF

Fig. 2 Radiation-wide association analyses (RWAS) reveal polygenic trait architectures and their admixture history.

(**a**) Spearman's ρ correlation coefficients between ecological and mating traits across all species in this study. Some pairs of traits show co-variation across species (highlighted with black squares; see text for details), but most traits do not covary strongly. (**b**) The repeatedly used component of genetic architecture of all traits is polygenic as demonstrated by the number of RWAS top SNPs significantly associated with each trait (top 99.99th percentile & PIP > 0.01 for binary/categorical traits and top 99.9th percentile & PIP > 0.01 for continuous traits; from here on denoted top SNPs). Little overlap of top SNPs among traits is interpreted as low pleiotropy among these traits (Hypergeometric test *p* values denote significant overlaps: $p < 0.001$***, $p < 0.01$**). (**c**) The absolute effect size (|β|) distribution of top SNPs for each trait shows some medium to larger effect SNPs and many small effect SNPs. (**d**) Most trait-associated top SNPs are also found in the closest living relatives of the Congolese and/or Nilotic ancestors of the Lake Victoria region superflock. Their origins hence predate not only the Lake Victoria cichlid radiation but the entire Lake Victoria Region species flock which includes radiations in Lakes Edward, Albert, Kivu and smaller satellite lakes. For each trait category we tested whether ancestral lineage SNPs are overrepresented among the trait-associated SNPs in the radiation (observed value exceeds 99th percentile of 100 iterations of randomly sampled SNPs across the genome; significantly enriched trait categories are indicated with an asterisk ($p < 0.05$*).

Genomic architecture of ecological and mating traits is modular

Our RWAS across 107 species revealed that the recurrently used components of genetic architectures of all 14 key traits were polygenic at the level of the radiation, varying in their complexity ranging from 18 to 147 significantly associated SNPs hereon denoted top SNPs per trait (top 99.99th percentile & PIP > 0.01 for binary/categorical traits and top 99.9th percentile & PIP > 0.01 for continuous traits (*65*)) (Fig. 2b). These top SNPs were dispersed across the genome for all traits (Fig. 3a, Fig. S3). Craniofacial shape and male nuptial colouration traits had the greatest number of top SNPs, followed by melanic stripe patterns, dentition and pharyngeal jaw morphology. With the RWAS approach it is likely that we detect fewer top SNPs for more complex and redundant trait architectures, with many alleles of small effect, different subsets of which could be used in each species (i.e. less recurrent use across the radiation). While traits with genetic architectures that are simple and repeatedly used across many species are statistically more likely to be detected. This is probably why we find more top SNPs for male nuptial colour motifs than for most ecomorphological traits (Fig. 2b). This is especially true for craniofacial shape, presumably a set of complex traits in itself that is summarised in dimension-reduced PC axes (Fig. 1, Fig. S1). The effect sizes of top SNPs varied within and between ecological and mating trait complexes (Fig. 2c). As expected, the presumably more quantitative traits

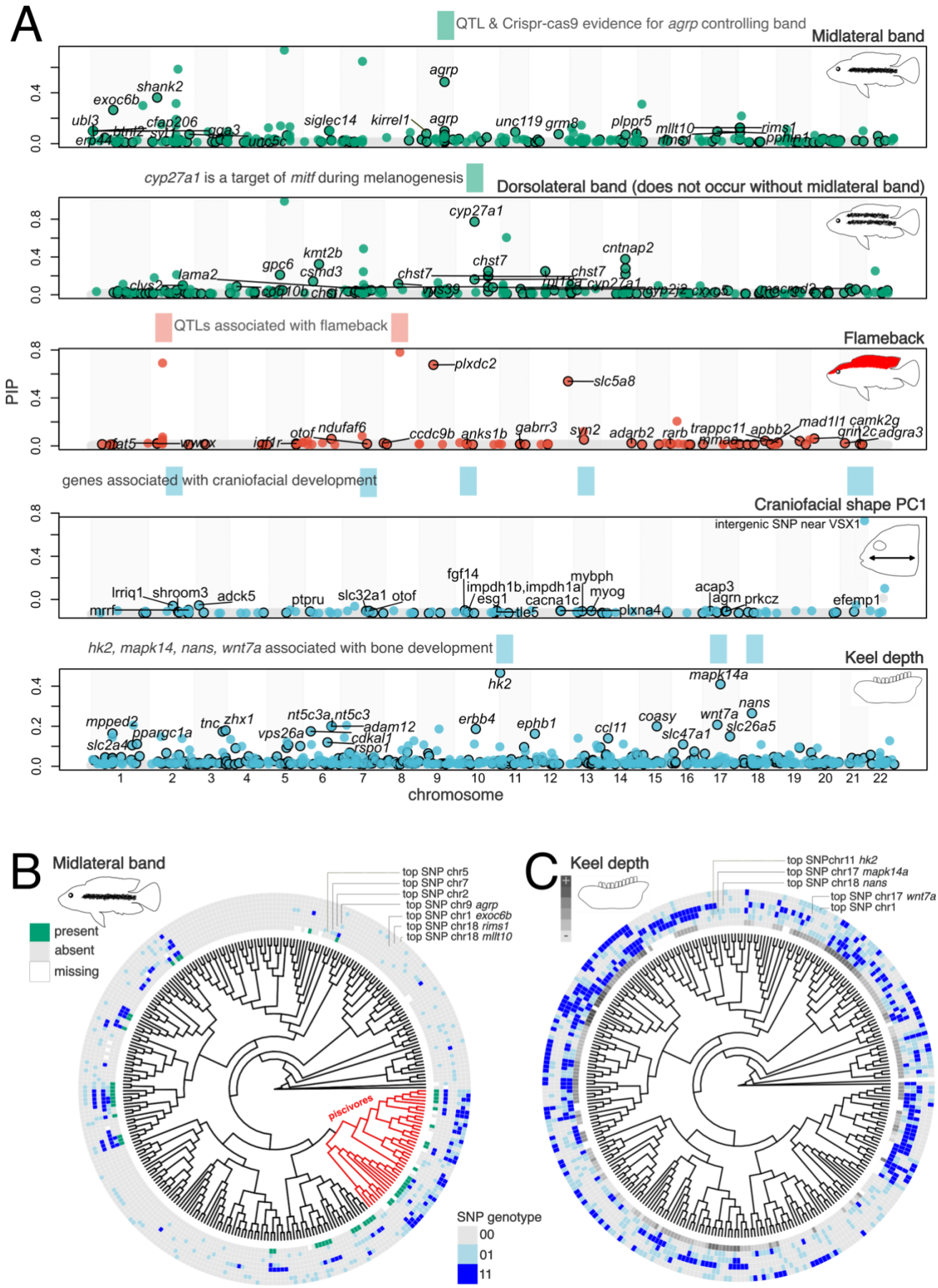
202 such as craniofacial shape PCs and pharyngeal keel depth were entirely determined by alleles of small effect,
203 while more categorical dentition traits such as the number of inner tooth rows in the oral jaw and cusp shape
204 of outer row oral teeth had some of the largest effect sizes. Interestingly, we found that the pharyngeal jaw
205 tooth molarisation was entirely determined by alleles of small effect, which is a sharp contrast to the simple
206 architecture of this trait in the polymorphic central American cichlid (*43*). Melanic stripe patterns and male
207 nuptial colour motifs were characterised by small to medium effect alleles (Fig. 2b). A detailed discussion of
208 candidate genes and GO enrichment (Fig. S4) for all traits can be found in the supplementary material. Next,
209 we applied random forest machine learning to evaluate the predictive accuracy of the top SNPs for the eight
210 binary body colour and patterning traits. Models were implemented within a five-fold cross-validation
211 scheme, where in each fold 80% of individuals were used for training and 20% were held out for testing.
212 Across traits, SNPs with higher PIP score were consistently associated with greater importance in phenotype
213 prediction models. Specifically, we observed significant positive Pearson correlations between SNP PIP
214 scores and their Mean Decrease in Accuracy (MDA) values from the Random Forest models for all traits
215 except Dorsolateral band (Fig. S5, Fig. S6). Correlation coefficients ranged from 0.31 to 0.78 ($p < 0.05$ for
216 each trait), indicating substantial concordance between RWAS-derived association strength and machine
217 learning–based predictive importance (Fig. S5).
218
219 Surprisingly, there was little overlap of top SNPs between traits (Fig. 2b) and this overlap did not increase
220 substantially even when we considered overlap at the level of genes containing top SNPs (Fig. S7). This
221 suggests low pleiotropy across the ecological and mating traits that are associated with the radiation, such that
222 variation in these traits can be largely independently inherited. Such modular architectures may have been
223 important for allowing many different trait combinations to evolve in very little time, permitting the
224 differentiation of this lineage into hundreds of different and phenotypically unique species. Those cases of
225 pleiotropy that we did find are consistent with what is known from patterns of co-variation across species in
226 nature and in species crosses in the lab, particularly among nuptial colour motifs and among stripe pattern
227 elements (Fig. 2a). For instance, the largest overlap were six top SNPs shared between Yellow flank (YF) and
228 Blue flank (BF) (hypergeometric test *p-value* < 0.001), which are divergent and mutually exclusive male
229 nuptial colours in the iconic *Pundamilia* sister species (Fig. 2b) and inversely correlated across the radiation
230 (Spearman $\rho = -0.8$; Fig. 2a). These six SNPs that had opposite effect sizes on these traits (+β on BF vs -β on
231 YF) mapped to *clul1*, a cone photoreceptor gene that is differentially expressed in light and dark-adapted
232 retinas (*66*), and *pdfgrb*, a vertebrate colouration gene(*67*). Male nuptial colouration and the ability to visually
233 detect it are expected to be correlated (*68*). Interestingly, we found *clul1* expressed in the eye transcriptomes
234 of *P. pundamilia* that has blue flank male nuptial colour and blue-shifted colour perception to live in light-

235 dark contrasting rock crevices in very shallow water (*68*). This gene was not expressed in the eye
236 transcriptome of its sister species *P. nyererei* that lives in deeper waters, has flameback and yellow-flank male
237 nuptial colour and red-shifted colour perception (*68*). It is possible that the top SNP which sits in the intron of
238 *clul1* may disrupt its expression. Other relevant cases of pleiotropy at the SNP and/or gene level were between
239 Midlateral band (MLB) and Dorsolateral band (DLB) – the latter is never expressed in Lake Victoria cichlids
240 without the former; Vertical bars (VB) and MLB – the occurrence of which are negatively correlated; and the
241 extent of lower pharyngeal jaw tooth molarisation and keel depth, which are strongly positively correlated
242 (*56*). In summary, the few trait correlations that we observe (Fig. 2a) seem at least partly caused by pleiotropic
243 gene action, but there is no evidence for pleiotropic gene action on other trait combinations.
244

245 **Trait modules are derived from ancestral hybridisation**

246 Despite the extraordinarily young age of the Lake Victoria cichlid fish radiation, its species have acquired
247 highly specialised trophic adaptations as well as a large array of nuptial colouration motifs and stripe patterns
248 that can be combined with each other and with trophic morphology in many ways (*56*, *69*, *70*). To better
249 understand the extent to which resuspension of ancient admixture variation through cycles of fusion and
250 fission processes (*9*) may have been important in enabling this process, we checked how many RWAS top
251 SNPs with statistical effects on craniofacial shape, dentition, pharyngeal jaw morphology, male nuptial colour
252 motifs, our body stripe patterns could be traced back to the extant Congolese and Nilotic haplochromine
253 species that are the closest known living relatives of those species that hybridised ~150,000 years ago to give
254 rise to the Lake Victoria region super-flock of radiations (*71*). Those same genetic variants must then also
255 have been cycled through the fission and fusion round associated with the period of total desiccation of Lake
256 Victoria and subsequent recolonisation by refugial populations 16,000 years ago (*9*). We found that up to
257 ~30% of the top RWAS alleles were unique to one of the ancestral lineages (Fig. 2d). Nilotic ancestor-derived
258 variants had significantly higher than expected contributions to genetic variation underlying all traits except
259 midlateral band (>99th percentile of 100 iterations of randomly sampled SNPs across the genome). Congolese
260 ancestor-derived variants had significantly higher than expected contributions to genetic variation underlying
261 all traits with the exception of red chest and craniofacial shape PC2 traits but including midlateral band. This
262 is notable because the midlateral band trait is found in the closest living relative of the Congolese ancestors
263 but not in those of the Nilotic ancestor (Fig. 2d fish drawings). Alleles of the *agouti-related protein* (*agrp*) in
264 particular were contributed to the LV radiation from their Congolese ancestors. The category of top alleles
265 that we could not trace to either of the ancestral lineages was not significantly overrepresented for any trait.
266 This suggests that the combination of ancestral genetic variants, combined into the ancestor of the radiation
267 through interspecific hybridisation, was important for the trait diversification that characterizes this radiation.

It is likely that some of the alleles that were carried into the ancestral hybrid swarm have since been lost in the closest living relatives of the ancestors that we sequenced, and we will have missed others in our limited sampling of these lineages compared to the large number of Lake Victoria cichlids that we sequenced. Hence, 30-50% of trait-associated alleles derived from admixture is likely an underestimate. The low pleiotropy among traits that we found would also be consistent with alleles undergirding those traits being old, selection-tested variants that have been swapped repeatedly among lineages through hybridization.


A
QTL & Crispr-cas9 evidence for agrp controlling band
Midlateral band
cyp27a1 is a target of mitf during melanogenesis
Dorsolateral band (does not occur without midlateral band)
QTLs associated with flameback
Flameback
PIP
genes associated with craniofacial development
Craniofacial shape PC1
intergenic SNP near VSX1
hk2, mapk14, nans, wnt7a associated with bone development
Keel depth
chromosome
B
Midlateral band
present
absent
missing
top SNP chr5
top SNP chr7
top SNP chr2
top SNP chr9 agrp
top SNP chr1 exoc6b
top SNP chr18 rims1
top SNP chr18 mllt10
piscivores
SNP genotype
00
01
11
C
Keel depth
top SNPchr11 hk2
top SNP chr17 mapk14a
top SNP chr18 nans
top SNP chr17 wnt7a
top SNP chr1

275 **Fig. 3 Genomically dispersed and redundant trait architectures in Lake Victoria cichlids.**
276 (**a**) RWAS top SNPs repeatedly associated with key adaptive radiation and speciation traits in the Lake
277 Victoria radiation are dispersed across the genome. Significant top SNPs are highlighted as coloured circles
278 and non-significant SNPs are denoted by grey circles (barely visible at the bottom of the Y axes). Colour of
279 circles are categorised by trait complexes, colour coded as in Fig. 2: blue – ecomorphology, green-melanic
280 stripe pattern, red-male nuptial colour. Coloured circles with black outline are SNPs located in genes and
281 coloured circles without black outline are SNPs located in intergenic regions. For each trait the top 20 genic
282 SNPs are annotated with gene names - full list can be found in File S1. Functional evidence linking candidate
283 genes to traits from previous QTL mapping and gene knock-out studies (cited in main and supplementary text)
284 for each trait is illustrated above each Manhattan plot (see Fig. S3 for all 14 key traits). (**b, c**) Two examples
285 of evolutionary genetic redundancy in key traits: midlateral and lower pharyngeal jaw keel depth repeatedly
286 evolved repeatedly across the LV radiation, but different top SNPs/genes are associated with the same trait in
287 different species and/or sub-clades in the radiation. Genetic redundancy plots for the other 12 traits are
288 presented in Fig. S8.
289

290 **Genetic redundancy contributed to rapid adaptive radiation**
291 Genetic redundancy is hypothesised to promote evolvability by providing multiple paths for similar
292 evolutionary change (*72–75*). Genetic redundancy has rarely been considered in the context of adaptive
293 radiation, but it can be a powerful mechanism to promote rapid repeated trait evolution during speciation. We
294 found evidence of genetic redundancy in trait architectures of all of the 14 repeatedly evolving traits in the
295 Lake Victoria cichlid radiation (Fig. 3b, Fig. S8). One noteworthy example was in the evolution of a
296 midlateral band (Fig. 3b). We mapped the top SNPs for midlateral band (Fig. 3b) across the phylogeny and
297 discovered that non-piscivorous species with midlateral band are almost always fixed for the alternative *agrp*
298 allele (G/G) but piscivore lineages, many species of which also have a midlateral band, do not carry this allele
299 (A/A) (Fig. 3b). The evolution of midlateral band may be driven by *exoc6b* in the piscivores, alleles of which
300 segregate almost perfectly with the presence of the midlateral band in heterozygous (C/G) or homozygous
301 state (G/G). *Exoc6b* plays a crucial role in the transfer of melanin from melanocytes to keratinocytes in *in*
302 *vitro* experiments(*76*). We next asked if genetic redundancy for this trait (and likely others) was generated by
303 the combination of ancestral gene pools into the hybrid swarm ancestor of the radiation. While we could map
304 the *agrp* G allele to the Congolese ancestor that possess a midlateral band, but the *exoc6b* G allele could not
305 be mapped to either the Nilotic or Congolese ancestor (File S1). This could be simply a consequence of small
306 sample size of ancestral lineages or it could reflect *de novo* evolution. We found similar patterns of genetic
307 redundancy among species with greater pharyngeal keel depths where *hk2/mapk14a* and *nans/wnt7a* appear to

function redundantly, but also additively in some species (Fig. 3c). Alleles for *hk2/mapk14a* and *nans* were present in both ancestral lineages but *wnt7a* allele was contributed exclusively by the Congolese ancestor (File S1). Given the repeated cycles of hybridisation in the ancestry of the Lake Victoria cichlid radiation (*9*, *71*), it is likely that genetically redundant alleles for the same traits arrived from different ancestral lineages from across the African continent (Fig. 2d). Such redundant genetic architectures emerging from hybridisation may be a previously underappreciated factor that provides a larger pool of genetic variation for repeated evolution of traits. This would promote the simultaneous and rapid evolution of many different species with different combinations of a limited set of phenotypic traits in this adaptive radiation.

Long-range linkage disequilibrium couples dispersed trait modules during combinatorial speciation

To test predictions of combinatorial speciation that we hypothesise to be responsible for the rapid emergence of several hundred distinct species in record time, we combined RWAS information on the genetic architecture and admixture history of recurrent allele use underlying major traits of adaptive radiation with estimates of the genomic landscape of speciation and species differentiation as inferred from genome wide divergence (F_{ST}) among closely related sympatric species. A unique prediction of the hypothesis of adaptive radiation through combinatorial speciation (combinatorial adaptive radiation) is that each species pair in the radiation is characterised by unique patterns of linkage disequilibrium (LD) among different subsets of reused trait-associated alleles (*51*). Here we set out to perform the first test of this prediction in any adaptive radiation. We calculated F_{ST} landscapes and determined differentiation outlier loci (top 5% F_{ST}) for 12 different sympatric pairs of closely related species in the Lake Victoria radiation. We then annotated F_{ST} outlier loci with top SNPs from our RWAS to identify in each species pair the subset of their 'speciation loci' (defined as loci displaying significantly stronger differentiation than the rest of the genome, in full sympatry; RWAS top SNPs that are also top 5% F_{ST}) and tested for LD among these loci. Firstly, we found that the genetic architecture of traits of speciation was oligogenic (File S1) and involved fewer loci than the polygenic global architecture of these same traits across the radiation (Fig. 2, File S1). Secondly, in most sympatric sister species contrasts, LD among their 'speciation loci' was significantly higher compared to their respective genomic background LD (wilcox.test $p < 0.001$) (Fig. 4a). There was a discernible build-up of LD in older species pairs such as *Pundamilia* on Makobe and Ruti islands compared to younger species pairs of *Pundamilia* on Python and Kissenda islands (Fig. 4a), that emerged much more recently through hybrid parallel speciation (*77*).

Next, we compared the distribution of these 'speciation loci' across the genome and LD between them for each species-pair (Fig. 4b, Fig. S10). We find that loci contributing to ecological traits and mate choice traits

are not physically linked in the genome but became coupled (presumably) during or shortly after speciation, with varying degrees of LD association among them. For example, between *P. nyererei* and *P. pundamilia* both trophic ecology (reef planktivore with fine pharyngeal bones and papilliform teeth vs crevice-dwelling insectivore with heavier pharyngeal bones and enlarged teeth) and male nuptial colour (flameback on yellow flank vs entirely blue flank) diverged, and this is reflected in the high LD between SNPs associated with these traits (Fig. 4b left). Similarly, *E. paropius* vs *E. coprologous* 'blue' are deeper water detritivores that diverged in male nuptial colouration (flameback on a light yellow flank vs blue flank with melanic underside), divergent melanic stripe patterns (present vs absent midlateral band), and subtle differences in trophic morphology (very fine pharyngeal bones and papilliform teeth vs slightly heavier pharyngeal bones and slightly enlarged teeth) which is reflected in LD among specific SNPs associated with these traits (Fig. 4b right). Long-range interchromosomal linkage disequilibrium between trait complexes in full sympatry suggests non-random mating among individuals with these divergent traits (i.e. speciation). Interestingly, we found that different and unique subsets of RWAS top SNPs had diverged in different species pairs, even among phenotypically similar species pairs (see *Pundamilia* species pairs from Makobe and Ruti versus Python and Kissenda, Fig. 4b, S7), reinforcing the idea that despite its extreme evolutionary youth, trait architectures can be characterised by redundant genotype-phenotype mapping in this radiation. The unique combination of LD patterns among subsets of trait-associated alleles in different species pairs that we observe provides support for the hypothesis of adaptive radiation through combinatorial speciation (*51*).

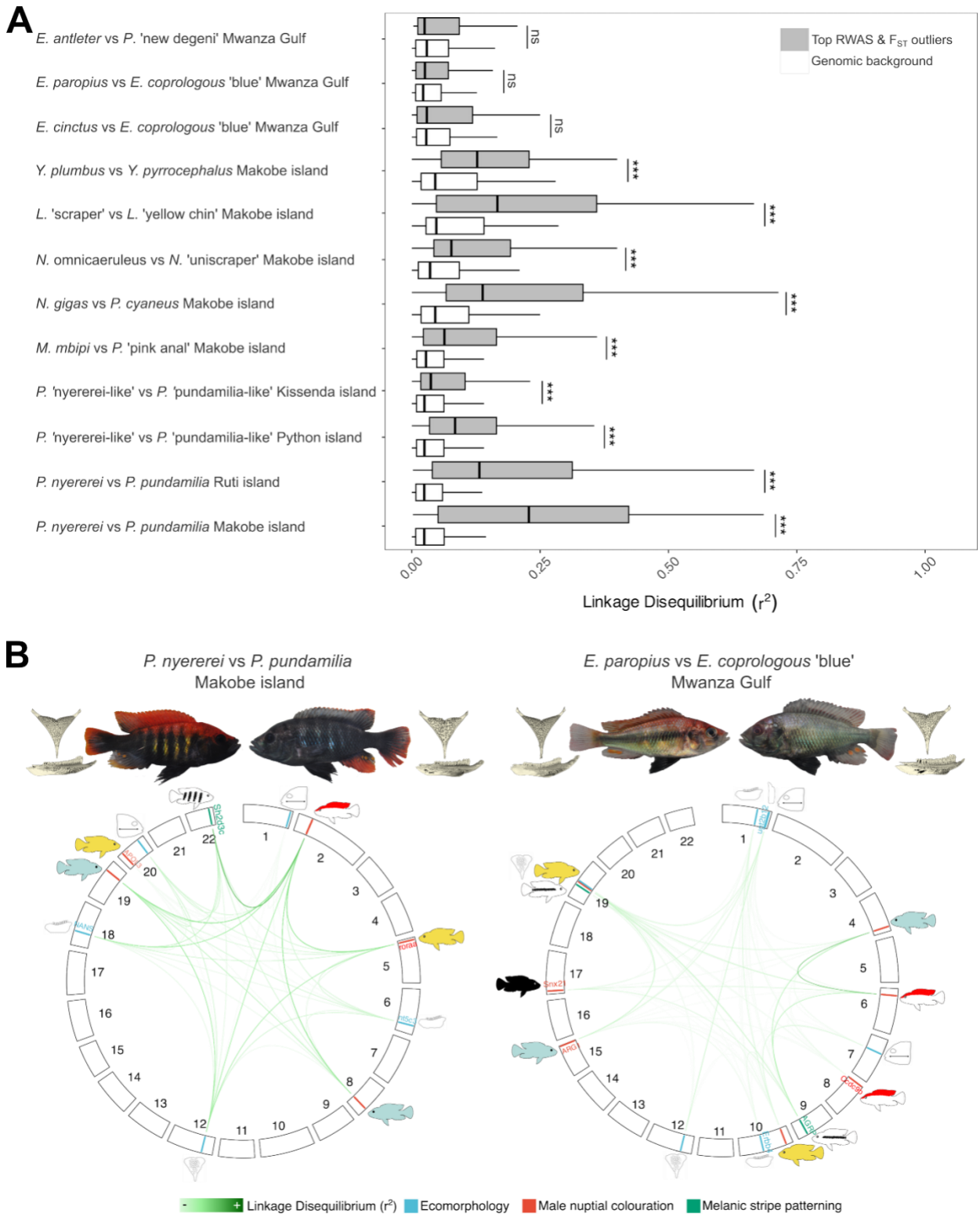


Fig. 4 Coupling of ecological and mating trait modules during combinatorial speciation. (**a**) Linkage disequilibrium (LD; measured using r^2 statistic) among RWAS top SNPs that are also top 5% F_{ST} (grey) outliers between closely related sympatric species compared to the genomic background LD (white).

363 LD among top SNP is significantly higher than background LD comparisons (wilcox.test $p < 0.001$***) for
364 all species pairs except demersal detritivores in the genus *Enterochromis*. (**b**) Genome-wide distribution of LD
365 coupled alleles for two sympatric species pairs, one where LD among top SNPs is significantly elevated and
366 one where it is not. It illustrates unique patterns of high interchromosomal LD among SNPs associated with
367 ecological and mating traits that diverge in both of these speciation events but divergent in unique
368 combinations among the species in each pair, consistent with expectation for combinatorial speciation from a
369 redundant variant pool. The plots for the remaining species pairs are shown in Fig. S9. Lines on the ideogram
370 represent a RWAS top SNPs that are also top 5% F_{ST} outliers; line colour denotes the trait complex the SNP
371 was associated with in the RWAS: blue = ecomorphology, green = melanic stripe pattern, red = male nuptial
372 colour motif as in Fig. 2; SNPs located in genes are annotated with gene names. Higher linkage disequilibrium
373 (r^2) values are depicted by darker green colours – see figure legend with scale. In all LD analyses only SNPs
374 on different chromosomes and SNPs more than 100 Kbp away on the same chromosome were included to
375 avoid effects of physical linkage. Even in the case of *Enterochromis* sister species where the LD among all top
376 SNPs is not significantly elevated over the genomic background, the highest LD patterns among top SNPs
377 map to trait combinations that differentiate these species.
378

379 **Discussion**

380 Paleontological research has shown that adaptive radiations have repeatedly punctuated the evolutionary
381 history of biodiversity on Earth in bursts of diversification that defy expectations of gradual evolution(*78*).
382 When adaptive radiation can proceed in the absence of geographical isolation among populations, it can in
383 principle be a rapidly acting modulator of biodiversity in moments when ecological opportunity arises, i.e.
384 when carrying capacity for species exceeds existing species diversity in a region (*79*). While this sometimes
385 happens, it more often does not, and the conditions for it to happen defy current understanding. In a classical
386 article back in 1954, Anderson & Stebbins proposed that such bursts of diversification can be enabled by
387 hybridisation between species (*10*), a proposition that received support from botanists but long-standing
388 criticism from zoologists (but see (*80*)). Yet zoologists have yet to resolve the puzzle of cichlid fish adaptive
389 radiations in African Great Lakes: how did hundreds of ecologically and morphologically diverse species arise
390 without geographic isolation and come to coexist in extraordinarily species rich assemblages within
391 sometimes just a few thousand years? Here, for the first time we empirically demonstrate that key ecological
392 and mating traits in this radiation correspond to independently inherited and often redundant genetic modules
393 derived from hybrid ancestry, which are leveraged in combinatorial speciation (*51*). Combining modules for
394 different traits in many different ways, like Lego bricks, or as Anderson & Stebbins (*10*) put it, elements from
395 assembly lines of several car models co-produced in the same factory, made it possible for so many diverse

species to evolve from a finite set of elements. We found that the large number of events of speciation in Lake Victoria in just a few thousand years are associated with the build-up and persistence in sympatry of long-range linkage disequilibrium among species-specific combinations of trait modules. Furthermore, we illustrate that genetic redundancy arising from parallel/convergent evolution of traits in the ancestors of the hybrid swarm and the subsequent combination of the parallel evolved architectures into a single hybrid population further increased flexibility for combinatorial speciation. We propose that modularity (independence) (*81*) in genomic architecture for different trait complexes that are key to ecological adaptation and mate choice was crucial for adaptive radiation to unfold at such a rapid pace through combinatorial speciation in the absence of geographical isolation.

Our association analysis revealed that all key traits associated with ecological diversification and speciation in the LV cichlid fish adaptive radiation are polygenic across the radiation and governed by a mix of some modest to larger effect alleles as well as many small effect alleles dispersed across the genome. However, while this pattern emerges across the radiation and is to a good extent due to genetic redundancy between species, divergence of the same traits in individual speciation events tends to be associated with only a subset of these genetic variants (Fig. 3b, Fig. S8, Fig. 4b, Fig. S9). This is also consistent with the observation that different trait-associated SNPs are recruited into high LD in different species pairs that diverge in the same set of traits (see flameback or pharyngeal keel depth in Fig 4b) and with the observation that the QTLs from laboratory crossing (*82*, *83*) are subsets of the variants associated with the corresponding traits in our radiation-wide association analysis. The compensatory phenotypic effects of genetically redundant alleles that we find add significantly to evolvability in key speciation traits in this adaptive radiation (*52*, *75*). The radiation-wide gene pool tends to hold multiple genetic solutions for species divergence in any particular combination of key traits through redundant genotype–phenotype mapping. Simulations have shown that polygenic and redundant trait architectures promote recurrent speciation and adaptive radiation in fusion-fission dynamics (*48*). Our analysis further revealed that the genetic architecture of speciation/species divergence is physically unlinked and non-pleiotropic i.e. it is modular (Fig. 4b, Fig. S9). It requires long range LD to build and then be maintained between multiple trait modules dispersed across the genome. This is consistent with earlier studies finding genome-wide divergence patterns already at very early stages of speciation in pairs of sympatric species (*22*, *84*). Taken together, we conclude that the global genetic architecture of key traits across the radiation is polygenic, but the genetic architecture of speciation is oligogenic. It is conceivable that moderate selection would be sufficient to maintain LD underlying oligogenic architectures during non-geographic speciation. In contrast, polygenic architectures would require strong selection on speciation traits, which we do not see evidence for in the field as strong selection would constrain

both speciation and trait diversity and speciation. Redundant genetic architectures of speciation may also allow emerging species to retain some genetic polymorphisms even for those traits that fixed under selection during speciation. Such context-dependent conversion of adaptive variation into cryptic variation and the reverse may facilitate successive speciation events in the same lineage involving divergence in (some of) the same trait sets.

The low pleiotropy and absence of physical linkage across adaptive radiation trait architectures may be surprising at first. Pleiotropic or clustered (i.e. physically linked by inversion polymorphisms) architectures were often suggested to facilitate speciation with gene flow. However, such architectures would constrain diversification beyond a finite number of ecotypes or species, as has been observed in sympatric and parapatric ecotypes of stickleback, and to some extent in Darwin's finches (*85–87*). The reason for this is that clustered architectures provide low evolutionary modularity – few components that can evolve independently without resulting in correlated effects on linked traits (*88*), and thus strong genetic constraints to the dimensionality of phenotypic evolution and low evolutionary flexibility. For the phenotypic complexity required for coexistence of large number of species through resource partitioning and mate choice to evolve rapidly, it seems advantageous if genetic variants affect only one trait at a time and are independently inherited (*89*). The modular nature of both mating and ecological traits that we demonstrated here for Lake Victoria cichlids may therefore be key to promoting diversification into many phenotypically diverse and unique species within the same and in different environments (*51*). This is consistent with classical theory on the importance of evolutionary modularity in promoting diversity in natural systems by circumnavigating constraints of selection on other traits (*90*). However, much of the empirical work on modularity is limited to morphological modularity and its role in diversification and adaptive radiation (*91–94*). Our study illustrates that both modularity in traits (illustrated by low phenotypic covariance among traits across the radiation (Fig 2a) and many different combinations of essentially the same set of craniofacial traits, dentition traits, body stripe patterns, and male nuptial colour motifs) and in their underlying genomic architecture is necessary for rapid diversification of a lineage into many sympatric species. The low pleiotropy among trait alleles that is required for this, may be explained by the enrichment of ancient admixture variation, originally derived from distantly related species that hybridized and then maintained through several fusion and fission cycles in the history leading to this radiation. Low pleiotropy would be unexpected had many of these trait-associated alleles had emerged from *de-novo* mutation within the radiation.

While we focused on SNPs here, it is very likely that small inversions or other small structural variants contribute additionally to modular trait architectures (*70*). In the cichlid radiation in Lake Malawi, large inversions that were exchanged between distantly related clades by hybridisation have been found to facilitate parallel adaptation to the same environmental gradients between these clades (*95*). While this explains parallel ecological transitions between major habitats it cannot explain how hundreds of ecologically diverse species arose within each major habitat. As we do not uncover any evidence for physical linkage among speciation and adaptive radiation trait modules in Lake Victoria cichlids, inversion polymorphisms that almost certainly segregate also in this radiation (*95*, *96*) do not appear to have captured major genetic variants that would physically couple key mate choice and adaptation traits in the radiation.

Questions that remain in order to understand the process of explosive adaptive radiation in Lake Victoria concern the nature of the evolutionary forces that generate and maintain various patterns of long-range linkage disequilibrium between genetic modules in the absence of geographical isolation. As important as the absence of strong genetic correlations between adaptive radiation traits is, the absence of strong ecological correlations between these traits, which we can also infer from our trait correlation matrix (Fig. 2A), is equally important. We argue this implies the absence of consistent selection constraint on trait associations. We show that trait values at ecological and mating traits can be combined in many ways and that some alleles associated with these two categories of traits in any specific sympatric species pair tend to be in elevated long-range LD. We interpret this as a signal of non-random mating among sympatric species, something for which good behavioural evidence also exists (*58*, *62*). In the presence of gene flow, the strength of phenotypic divergent selection required on a trait or trait combination to maintain LD increases with the number of loci involved in phenotypic divergence which can be at least partly offset by non-random mating (*97*). Oligogenic speciation architectures as observed here might require only very moderate strengths of selection in the face of non-random mating. Clearly, LD among multiple traits, many involving more than one region in the genome, including traits that are associated with mate choice, persists in sympatry without physical linkage. Future work will have to address how it arises from a situation of no LD (or very different LD) in the first place. One possibility is initial build-up of a novel pattern of LD among ecological and mating traits happens in transient geographical or ecological parapatry, and that the extent of assortative mating generated in this way becomes quickly sufficient for incipient species to persist as they reinvade each other's ranges and form sympatric species communities. Our findings reconcile quantitative genetics of traits with population genomics of speciation and have broad implications for understanding conditions under which adaptive radiation can act as a modulator of biodiversity when ecological opportunity arises. With the genomic architecture of species divergence in ecological and mating traits for many species revealed, future work can address the remaining

494 questions, such as the evolutionary forces driving LD patterns, through combining ecological and genomics
495 work at the scale of entire assemblages of species.
496

497 **References**

498 1. P. Neige, *Events of Increased Biodiversity* (ISTE Press - Elsevier, 2015).
499 2. G. G. Simpson, *The Major Features of Evolution* (Columbia University Press, Ney York, 1953).
500 3. G. G. Simpson, *Tempo and Mode in Evolution* (Columbia University Press, New York, 1944).
501 4. R. G. Gillespie, G. M. Bennett, L. De Meester, J. L. Feder, R. C. Fleischer, L. J. Harmon, A. P.
502 Hendry, M. L. Knope, J. Mallet, C. Martin, C. E. Parent, A. H. Patton, K. S. Pfennig, D. Rubinoff, D.
503 Schluter, O. Seehausen, K. L. Shaw, E. Stacy, M. Stervander, J. T. Stroud, C. Wagner, G. O. U.
504 Wogan, Comparing adaptive radiators across space, time and taxa. *Journal of Heredity* **89154**, 14853
505 (2020).
506 5. R. De-Kayne, R. Schley, J. M. I. Barth, L. Campillo, C. Chaparro-Pedraza, J. Joshi, W. Salzburger, B.
507 Van Bocxlaer, D. Cotoras, C. Fruciano, Why do some lineages radiate while others do not?
508 Perspectives for future research on adaptive radiations. *Cold Spring Harb. Perspect. Biol.*, doi:
509 10.1101/cshperspect.a041448ï (20204).
510 6. J. B. Losos, Adaptive radiation, ecological opportunity, and evolutionary determinism. *Am. Nat.* **175**,
511 623–639 (2010).
512 7. J. W. Poelstra, E. J. Richards, C. H. Martin, Speciation in sympatry with ongoing secondary gene flow
513 and a potential olfactory trigger in a radiation of Cameroon cichlids. *Mol. Ecol.* **27**, 4270–4288 (2018).
514 8. C. J. Rubin, E. D. Enbody, M. P. Dobreva, A. Abzhanov, B. W. Davis, S. Lamichhaney, M. Pettersson,
515 A. T. Sendell-Price, C. G. Sprehn, C. A. Valle, K. Vasco, O. Wallerman, B. R. Grant, P. R. Grant, L.
516 Andersson, Rapid adaptive radiation of Darwin's finches depends on ancestral genetic modules. *Sci.*
517 *Adv.* **8**, 5982 (2022).
518 9. J. I. Meier, M. D. McGee, D. A. Marques, S. Mwaiko, M. Kishe, S. Wandera, D. Neumann, H.
519 Mrosso, L. J. Chapman, C. A. Chapman, L. Kaufman, A. Taabu-Munyaho, C. E. Wagner, R.
520 Bruggmann, L. Excoffier, O. Seehausen, Cycles of fusion and fission enabled rapid parallel adaptive
521 radiations in African cichlids. *Science (1979).* **381** (2023).
522 10. E. Anderson, G. L. Stebbins Jr., Hybridization as an evolutionary stimulus. *Evolution (N Y).* **8**, 378–
523 388 (1954).
524 11. G. Gaylord Simpson, *Evolution and Geography* (Condon Lecure Publications, Singapore, 1953).

12. C. H. Martin, E. J. Richards, The Paradox behind the Pattern of Rapid Adaptive Radiation: How Can the Speciation Process Sustain Itself Through an Early Burst? *Annu. Rev. Ecol. Evol. Syst.* **50**, 569–593 (2019).

13. I. Irisarri*, P. Singh*, S. Koblmüller, J. Torres-Dowdall, F. Henning, P. Franchini, C. Fischer, A. R. Lemmon, E. M. Lemmon, G. G. Thallinger, C. Sturmbauer, A. Meyer, Phylogenomics uncovers early hybridization and adaptive loci shaping the radiation of Lake Tanganyika cichlid fishes. *Nat. Commun.* **9**, 3159 (2018).

14. H. Svardal, F. X. Quah, M. Malinsky, B. P. Ngatunga, E. A. Miska, W. Salzburger, M. J. Genner, G. F. Turner, R. Durbin, Ancestral Hybridization Facilitated Species Diversification in the Lake Malawi Cichlid Fish Adaptive Radiation. *Mol. Biol. Evol.* **37**, 1100–1113 (2020).

15. R. L. Moran, J. B. Jaggard, E. Y. Roback, A. Kenzior, N. Rohner, J. E. Kowalko, C. P. Ornelas-García, S. E. McGaugh, A. C. Keene, Hybridization underlies localized trait evolution in cavefish. *iScience* **25**, 103778 (2022).

16. M. P. K. Blom, V. Peona, S. Prost, L. Christidis, B. W. Benz, K. A. Jønsson, A. Suh, M. Irestedt, Hybridization in birds-of-paradise: Widespread ancestral gene flow despite strong sexual selection in a lek-mating system. *iScience* **27**, 110300 (2024).

17. S. Hübner, N. Bercovich, M. Todesco, J. R. Mandel, J. Odenheimer, E. Ziegler, J. S. Lee, G. J. Baute, G. L. Owens, C. J. Grassa, D. P. Ebert, K. L. Ostevik, B. T. Moyers, S. Yakimowski, R. R. Masalia, L. Gao, I. Ćalić, J. E. Bowers, N. C. Kane, D. Z. H. Swanevelder, T. Kubach, S. Muños, N. B. Langlade, J. M. Burke, L. H. Rieseberg, Sunflower pan-genome analysis shows that hybridization altered gene content and disease resistance. *Nature Plants 2018 5:1* **5**, 54–62 (2018).

18. T. N. Nguyen, M. Repenning, C. S. Fontana, L. Campagna, Genomic islands of speciation harbor genes underlying coloration differences in a pair of Neotropical seedeaters. *Evolution (N Y).* **78**, 1161–1173 (2024).

19. S. Lamichhaney, J. Berglund, M. S. Almén, K. Maqbool, M. Grabherr, A. Martinez-Barrio, M. Promerová, C.-J. Rubin, C. Wang, N. Zamani, B. R. Grant, P. R. Grant, M. T. Webster, L. Andersson, Evolution of Darwin's finches and their beaks revealed by genome sequencing. *Nature*, doi: 10.1038/nature14181 (2015).

20. N. J. Nadeau, A. Whibley, R. T. Jones, J. W. Davey, K. K. Dasmahapatra, S. W. Baxter, M. A. Quail, M. Joron, R. H. Ffrench-Constant, M. L. Blaxter, J. Mallet, C. D. Jiggins, Genomic islands of divergence in hybridizing Heliconius butterflies identified by large-scale targeted sequencing. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**, 343–353 (2012).

21. H. A. Hejase, A. Salman-Minkov, L. Campagna, M. J. Hubisz, I. J. Lovette, I. Gronau, A. Siepel, Genomic islands of differentiation in a rapid avian radiation have been driven by recent selective sweeps. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 30554–30565 (2020).

22. J. I. Meier, D. A. Marques, C. E. Wagner, L. Excoffier, O. Seehausen, Genomics of parallel ecological speciation in Lake Victoria cichlids. *Mol. Biol. Evol.* **35**, 1489–1506 (2018).

23. O. Seehausen, R. K. Butlin, I. Keller, C. E. Wagner, J. W. Boughman, P. a Hohenlohe, C. L. Peichel, G.-P. Saetre, C. Bank, A. Brännström, A. Brelsford, C. S. Clarkson, F. Eroukhmanoff, J. L. Feder, M. C. Fischer, A. D. Foote, P. Franchini, C. D. Jiggins, F. C. Jones, A. K. Lindholm, K. Lucek, M. E. Maan, D. a Marques, S. H. Martin, B. Matthews, J. I. Meier, M. Möst, M. W. Nachman, E. Nonaka, D. J. Rennison, J. Schwarzer, E. T. Watson, A. M. Westram, A. Widmer, Genomics and the origin of species. *Nat. Rev. Genet.* **15**, 176–92 (2014).

24. D. A. Marques, F. C. Jones, F. Di Palma, D. M. Kingsley, T. E. Reimchen, Genomic changes underlying repeated niche shifts in an adaptive radiation. *Evolution (N Y).* **76**, 1301–1319 (2022).

25. A. F. Kautt, C. F. Kratochwil, A. Nater, G. Machado-Schiaffino, M. Olave, F. Henning, J. Torres-Dowdall, A. Härer, C. D. Hulsey, P. Franchini, M. Pippel, E. W. Myers, A. Meyer, Contrasting signatures of genomic divergence during sympatric speciation. *Nature* **588** (2020).

26. R. Riesch, M. Muschick, D. Lindtke, R. Villoutreix, A. A. Comeault, T. E. Farkas, K. Lucek, E. Hellen, V. Soria-Carrasco, S. R. Dennis, C. F. De Carvalho, R. J. Safran, C. P. Sandoval, J. Feder, R. Gries, B. J. Crespi, G. Gries, Z. Gompert, P. Nosil, Transitions between phases of genomic differentiation during stick-insect speciation. *Nature Ecology & Evolution 2017 1:4* **1**, 0082- (2017).

27. C. Dufresnes, A. Brelsford, D. L. Jeffries, G. Mazepa, T. Suchan, D. Canestrelli, A. Nicieza, L. Fumagalli, S. Dubey, I. Martínez-Solano, S. N. Litvinchuk, M. Vences, N. Perrin, P. A. Crochet, Mass of genes rather than master genes underlie the genomic architecture of amphibian speciation. *Proc. Natl. Acad. Sci. U. S. A.* **118**, e2103963118 (2021).

28. M. Malinsky, R. J. Challis, A. M. Tyers, S. Schiffels, Y. Terai, B. P. Ngatunga, E. A. Miska, R. Durbin, M. J. Genner, G. F. Turner, Genomic islands of speciation separate cichlid ecomorphs in an East African crater lake. *Science (1979).* **350**, 1493–1498 (2015).

29. K. Hench, M. Vargas, M. P. Höppner, W. O. McMillan, O. Puebla, Inter-chromosomal coupling between vision and pigmentation genes during genomic divergence. *Nature Ecology & Evolution 2019 3:4* **3**, 657–667 (2019).

30. K. Hench, M. Helmkampf, W. O. McMillan, O. Puebla, Rapid radiation in a highly diverse marine environment. *Proc. Natl. Acad. Sci. U. S. A.* **119** (2022).

31. L. Campagna, M. Repenning, L. F. Silveira, C. S. Fontana, P. L. Tubaro, I. J. Lovette, Repeated divergent selection on pigmentation genes in a rapid finch radiation. *Sci. Adv.* **3** (2017).
32. S. M. Van Belleghem, P. Rastas, A. Papanicolaou, S. H. Martin, C. F. Arias, M. A. Supple, J. J. Hanly, J. Mallet, J. J. Lewis, H. M. Hines, M. Ruiz, C. Salazar, M. Linares, G. R. P. Moreira, C. D. Jiggins, B. A. Counterman, W. O. McMillan, R. Papa, Complex modular architecture around a simple toolkit of wing pattern genes. *Nature Ecology & Evolution 2017 1:3* **1**, 0052- (2017).
33. K. F. Stryjewski, M. D. Sorenson, Mosaic genome evolution in a recent and rapid avian radiation. *Nature Ecology & Evolution 2017 1:12* **1**, 1912–1922 (2017).
34. P. Nosil, L. J. Harmon, O. Seehausen, Ecological explanations for (incomplete) speciation. *Trends Ecol. Evol.* **24**, 145–156 (2009).
35. M. Kirkpatrick, N. Barton, Chromosome Inversions, Local Adaptation and Speciation. *Genetics* **173**, 419–434 (2006).
36. S. Yeaman, M. C. Whitlock, The genetic architecture of adaptation under migration-selection balance. *Evolution (N Y).* **65**, 1897–1911 (2011).
37. M. R. Servedio, G. S. Van Doorn, M. Kopp, A. M. Frame, P. Nosil, Magic traits in speciation: “magic” but not rare? *Trends Ecol. Evol.* **26**, 389–397 (2011).
38. M. Kopp, M. R. Servedio, T. C. Mendelson, R. J. Safran, R. L. Rodríguez, M. E. Hauber, E. C. Scordato, L. B. Symes, C. N. Balakrishnan, D. M. Zonana, G. S. Van Doorn, Mechanisms of assortative mating in speciation with gene flow: Connecting theory and empirical research. *American Naturalist* **191**, 1–20 (2018).
39. S. Gavrilets, *Fitness Landscapes and the Origin of Species* (Princeton University Press, 2004).
40. J. Felsenstein, Skepticism Towards Santa Rosalia, or Why are There so Few Kinds of Animals? *Evolution (N Y).* **35**, 124 (1981).
41. G. F. Kathleen, L. L. Barnett, B. K. Blackman, J. H. Willis, Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature 2001 412:6850* **412**, 904–907 (2001).
42. K. G. Ferris, L. L. Barnett, B. K. Blackman, J. H. Willis, The genetic architecture of local adaptation and reproductive isolation in sympatry within the Mimulus guttatus species complex. *Mol. Ecol.* **26**, 208–224 (2017).
43. C. D. Hulsey, P. Franchini, P. Masonick, A. Kautt, G. Machado-Schiaffino, M. Pippel, F. J. García-de León, E. Myers, A. Meyer, Divergence at the IRX gene cluster underlies extreme trophic polymorphism in a cichlid fish (Herichthys minckleyi). *Communications Biology 2026*, doi: 10.1038/s42003-026-09689-6 (2026).

44. P. Singh, M. Taborsky, C. L. Peichel, C. Sturmbauer, Genomic basis of Y-linked dwarfism in cichlids pursuing alternative reproductive tactics. *Mol. Ecol.* **32**, 1592–1607 (2023).
45. S. Yeaman, Local adaptation by alleles of small effect. *American Naturalist* **186**, S74–S89 (2015).
46. S. Flaxman, A. C. Wacholder, J. L. Feder, P. Nosil, Theoretical models of the influence of genomic architecture on the dynamics of speciation. *Mol. Ecol.* **23**, 4074–4088 (2014).
47. K. Kagawa, G. Takimoto, Hybridization can promote adaptive radiation by means of transgressive segregation. *Ecol. Lett.*, doi: 10.1111/ele.12891 (2017).
48. K. Kagawa, O. Seehausen, The propagation of admixture-derived adaptive radiation potential. *Proceedings of the Royal Society B: Biological Sciences* **287** (2020).
49. S. Gavrilets, A. Vose, Dynamic patterns of adaptive radiation. *PNAS* **102**, 18040–18045 (2005).
50. S. Gavrilets, J. B. Losos, Adaptive radiation: contrasting theory with data. *Science (1979).* **323**, 732–737 (2009).
51. D. A. Marques, J. I. Meier, O. Seehausen, A combinatorial view on speciation and adaptive radiation. *Trends Ecol. Evol.* **34**, 531–544 (2019).
52. N. Barghi, J. Hermisson, C. Schlötterer, Polygenic adaptation: a unifying framework to understand positive selection. *Nature Reviews Genetics 2020 21:12* **21**, 769–781 (2020).
53. O. Seehausen, P. J. Mayhew, J. J. M. Van Alphen, Evolution of colour patterns in East African cichlid fish. *J. Evol. Biol.* **12**, 514–534 (1999).
54. O. Seehausen, J. J. M. Van Alphen, Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria? *Ecol. Lett.* **2**, 262–271 (1999).
55. O. Seehausen, J. J. M. Van Alphen, F. Witte, Can ancient colour polymorphisms explain why some cichlid lineages speciate rapidly under disruptive sexual selection? *Belg. J. Zool.* **129**, 43–60 (1999).
56. O. Seehausen, *Lake Victoria Rock Cichlids* (Verduyn Cichlids, 1996).
57. B. Barel, M. van Oijen, F. Witte, E. Witte-Maas, An introduction to the taxonomy and morphology of the haplochromine cichlidae from Lake Victoria. *Netherlands Journal of Zoology* **27**, 333–380 (1976).
58. O. M. Selz, M. E. R. Pierotti, M. E. Maan, C. Schmid, O. Seehausen, Female preference for male color is necessary and sufficient for assortative mating in 2 cichlid sister species. *Behavioral Ecology* **25**, 612–626 (2014).
59. P. Dijkstra, O. Seehausen, T. Groothuis, Direct male-male competition can facilitate invasion of new colour types in Lake Victoria cichlids. *Behav Ecol Sociobiol* **58**, 136–143 (2005).
60. O. Svensson, K. Woodhouse, A. Smith, O. Seehausen, G. F. Turner, Sympatry and parapatry among rocky reef cichlids of Lake Victoria explained by female mating preferences. *J. Evol. Biol.* **37**, 51–61 (2024).

61. O. Seehausen, D. Schluter, Male-male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proceedings of the Royal Society B: Biological Sciences* **271**, 1345–1353 (2004).

62. O. Seehausen, F. Witte, J. J. M. Van Alphen, N. Bouton, Direct mate choice maintains diversity among sympatric cichlids in Lake Victoria. *J. Fish Biol.* **53**, 37–55 (1998).

63. O. Seehausen, J. J. Van Alphen, F. Witte, Can ancient colour polymorphisms explain why some cichlid lineages speciate rapidly under disruptive sexual selection? *Belg. J. Zool.* **129**, 43–60 (1999).

64. S. J. Arnold, R. Bürger, P. A. Hohenlohe, B. C. Ajie, A. G. Jones, Understanding the Evolution and Stability of the G-Matrix. *Evolution* **62**, 2451 (2008).

65. A. A. Comeault, V. Soria-Carrasco, Z. Gompert, T. E. Farkas, C. A. Buerkle, T. L. Parchman, P. Nosil, Genome-wide association mapping of phenotypic traits subject to a range of intensities of natural selection in Timema cristinae. *American Naturalist* **183**, 711–727 (2014).

66. Q. Zhang, W. A. Beltran, Z. Mao, K. Li, J. L. Johnson, G. M. Acland, G. D. Aguirre, Comparative Analysis and Expression of CLUL1, a Cone Photoreceptor-Specific Gene. *Invest. Ophthalmol. Vis. Sci.* **44**, 4542–4549 (2003).

67. I. Braasch, W. Salzburger, A. Meyer, Asymmetric evolution in two fish-specifically duplicated receptor tyrosine kinase paralogons involved in teleost coloration. *Mol. Biol. Evol.* **23**, 1192–1202 (2006).

68. O. Seehausen, Y. Terai, I. S. Magalhaes, K. L. Carleton, H. D. J. Mrosso, R. Miyagi, I. Van Der Sluijs, M. V. Schneider, M. E. Maan, H. Tachida, H. Imai, N. Okada, Speciation through sensory drive in cichlid fish. *Nature* **455**, 620–626 (2008).

69. P. H. Greenwood, The cichlid fishes of Lake Victoria, East Africa: the biology and evolution of a species flock. *Bulletin of the British Museum (Natural History). Zoology* **Supplement 6**, 1--134 (1974).

70. M. D. McGee, S. R. Borstein, J. I. Meier, D. A. Marques, S. Mwaiko, A. Taabu, M. A. Kishe, B. O'Meara, R. Bruggmann, L. Excoffier, O. Seehausen, The ecological and genomic basis of explosive adaptive radiation. *Nature* **586**, 75–79 (2020).

71. J. I. Meier, D. A. Marques, S. Mwaiko, C. E. Wagner, L. Excoffier, O. Seehausen, Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nat. Commun.* **8**, 14363 (2017).

72. R. Kafri, M. Springer, Y. Pilpel, Genetic Redundancy: New Tricks for Old Genes. *Cell* **136**, 389–392 (2009).

73. K. Bomblies, Evolution: redundancy as an opportunity for innovation. *Current Biology* **20**, R320–R322 (2010).

74. Á. J. Láruson, S. Yeaman, K. E. Lotterhos, The Importance of Genetic Redundancy in Evolution. doi: 10.1016/j.tree.2020.04.009 (2020).

75. M. A. Nowak, M. C. Boerlijst, J. Cooke, J. M. Smith, Evolution of genetic redundancy. *Nature 1997 388:6638* **388**, 167–171 (1997).

76. H. Moreiras, F. J. C. Pereira, M. V. Neto, L. Bento-Lopes, T. C. Festas, M. C. Seabra, D. C. Barral, The exocyst is required for melanin exocytosis from melanocytes and transfer to keratinocytes. *Pigment Cell Melanoma Res.* **33**, 366–371 (2020).

77. J. I. Meier, V. C. Sousa, D. A. Marques, O. M. Selz, C. E. Wagner, L. Excoffier, O. Seehausen, Demographic modelling with whole-genome data reveals parallel origin of similar Pundamilia cichlid species after hybridization. *Mol. Ecol.* **26**, 123–141 (2017).

78. N. Eldredge, S. J. Gould, "Punctuated equilibria: an alternative to phyletic gradualism" in *Models in Paleobiology*, T. F. Schopf, Ed. (Cooper & Co, San Francisco, 1972), pp. 82–115.

79. D. Schluter, *The Ecology of Adaptive Radiation* (Oxford University Press, Oxford, 2000).

80. O. Seehausen, Hybridization and adaptive radiation. *Trends Ecol. Evol.* **19**, 198–207 (2004).

81. G. P. Wagner, M. Pavlicev, J. M. Cheverud, The road to modularity. *Nat. Rev. Genet.* **8**, 921–931 (2007).

82. A. F. Feller, O. Seehausen, Genetic architecture of adaptive radiation across two trophic levels. *Proceedings of the Royal Society B: Biological Sciences* **289**, 20220377 (2022).

83. A. F. Feller, M. P. Haesler, C. L. Peichel, O. Seehausen, Genetic architecture of a key reproductive isolation trait differs between sympatric and non-sympatric sister species of Lake Victoria cichlids. *Proceedings of the Royal Society B: Biological Sciences* **287** (2020).

84. D. A. Marques, K. Lucek, J. I. Meier, S. Mwaiko, C. E. Wagner, L. Excoffier, O. Seehausen, Genomics of rapid incipient speciation in sympatric threespine stickleback. *PLoS Genet.* **12**, e1005887 (2016).

85. F. C. Jones, M. G. Grabherr, Y. F. Chan, P. Russell, E. Mauceli, J. Johnson, R. Swofford, M. Pirun, M. C. Zody, S. White, E. Birney, S. Searle, J. Schmutz, J. Grimwood, M. C. Dickson, R. M. Myers, C. T. Miller, B. R. Summers, A. K. Knecht, S. D. Brady, H. Zhang, A. A. Pollen, T. Howes, C. Amemiya, J. Baldwin, T. Bloom, D. B. Jaffe, R. Nicol, J. Wilkinson, E. S. Lander, F. Di Palma, K. Lindblad-Toh, D. M. Kingsley, The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* **484**, 55–61 (2012).

86. J. B. W. Wolf, H. Ellegren, Making sense of genomic islands of differentiation in light of speciation. *Nature Publishing Group*, doi: 10.1038/nrg.2016.133 (2016).

87. E. D. Enbody, A. T. Sendell-Price, C. G. Sprehn, C. J. Rubin, P. M. Visscher, B. R. Grant, P. R. Grant, L. Andersson, Community-wide genome sequencing reveals 30 years of Darwin's finch evolution. *Science* **381**, eadf6218 (2023).
88. D. Melo, A. Porto, J. M. Cheverud, G. Marroig, Modularity: Genes, development, and evolution. *Annu. Rev. Ecol. Evol. Syst.* **47**, 463–486 (2016).
89. H. A. Orr, Adaptation and the cost of complexity. *Evolution* **54**, 13–20 (2000).
90. G. P. Wagner, L. Altenberg, Perspective: Complex Adaptations and the Evolution of Evolvability. *Evolution (N Y).* **50**, 967 (1996).
91. M. P. Neves, H. W. Chan, E. C. M. Santos, K. M. Evans, Cranial modularity drives phenotypic diversification and adaptive radiation of Antarctic icefishes. *Proc. Natl. Acad. Sci. U. S. A.* **122**, e2503283122 (2025).
92. A. S. Yang, Modularity, evolvability, and adaptive radiations: a comparison of the hemi- and holometabolous insects. *Evol. Dev.* **3**, 59–72 (2001).
93. M. D. Burns, M. L. Collyer, B. L. Sidlauskas, Simultaneous integration and modularity underlie the exceptional body shape diversification of characiform fishes. *Evolution (N Y).* **77**, 746–762 (2023).
94. C. P. Klingenberg, Morphological integration and developmental modularity. *Annu. Rev. Ecol. Evol. Syst.* **39**, 115–132 (2008).
95. L. M. Blumer, V. Burskaia, I. Artiushin, J. Saha, J. C. Garcia, F. C. Jiménez, A. Hooft van der Huysdynen, J. Elkin, B. Fischer, N. Van Houtte, C. Zhou, S. Gresham, M. Malinsky, T. Linderoth, W. Sawasawa, G. Vernaz, I. Bista, A. Hickey, M. Kucka, S. Louzada, R. Zatha, F. Yang, B. Rusuwa, M. E. Santos, Y. F. Chan, D. A. Joyce, A. Böhne, E. A. Miska, M. Ngochera, G. F. Turner, R. Durbin, H. Svardal, Introgression dynamics of sex-linked chromosomal inversions shape the Malawi cichlid radiation. *Science (1979).* **388**, eadr9961 (2025).
96. A. F. Feller, V. Ogi, O. Seehausen, J. I. Meier, Identification of a novel sex determining chromosome in cichlid fishes that acts as XY or ZW in different lineages. *Hydrobiologia* **848**, 3727–3745 (2021).
97. M. Kirkpatrick, V. Ravigné, Speciation by natural and sexual selection: models and Experiments. *Am. Nat.* **159**, S22–S35 (2002).
98. D. A. Marques, J. I. Meier, M. D. McGee, M. P. Haesler, S. Mwaiko, M. A. Kishe, A. Taabu-Munyaho, L. Chapman, C. A. Chapman, S. B. Wandera, C. E. Wagner, L. E. Excoffier, O. Seehausen, Genomes reveal age and demographic consequence of ultrafast adaptive radiation. *bioRxiv*, 2025.03.07.638630 (2025).

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Author contribution

Pooja Singh: conception, lead all analyses and interpretation, and manuscript writing. Heidi Tschanz-Lischer: genome assembly and annotation. Kassandra Ford: 3D morphometrics data collection and analyses. Ehsan P. Ahi: candidate gene & GO analysis interpretation. Marcel Haesler: fish husbandry and tissue dissections. Salome Mwaiko: DNA and RNA extraction. Joana I. Meier: DNA extraction. David Marques: conception. Remy Bruggman: genome assembly support. Mary Kishe: fieldwork. Ole Seehausen: fish collection and identification, conception, interpretation and manuscript writing.

Competing interests

Authors declare that they have no competing interests.

Data, code, and materials availability

Pundamilia nyererei v3 genome assembly and annotation and the raw PacBio Hi-C and PacBio Iso-seq data is deposited to the NCBI SRA (BioProject PRJNA1231206). Individual resequencing data for 313 individuals is sourced from (*9*, *70*, *98*), with the exception of 11 newly sequencing sampled that have been deposited to NCBI SRA BioProject PRJNA1231206. All sample and phenotyping information is in File S1. 3D scans have been deposited to BioImageArchive (doi: 10.6019/S-BIAD2928). Scripts used in this study are available on Github: https://github.com/poojasingh09/2025_singh_radiation_gwas.

Supplementary Materials

Materials and Methods

Supplementary Text

Table S1

Figures S1 – S11

File S1