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Variation in parasite infection between replicates of speciation in Lake Victoria cichlid fish

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Abstract

Because of potentially strong eco-evolutionary interactions with their hosts, parasites may initiate or enhance host diversification. The adaptive radiation of cichlid fish in Lake Victoria provides a good system to study the role of parasites at different stages of host speciation. We analyzed the macroparasite infection of four replicates of sympatric blue and red *Pundamilia* species pairs that vary in their age and extent of differentiation. Sympatric host species differed in parasite community composition and in the infection levels of some of these parasite taxa. Most infection differences were consistent between sampling years, indicating temporal consistency in parasite-mediated divergent selection between species. Infection differentiation increased linearly with genetic differentiation. However, significant infection differences between sympatric species were only found in the oldest, most strongly differentiated *Pundamilia* species pair. This is inconsistent with parasite-driven speciation. Next, we identified five distinct species of *Cichlidogyrus*, a genus of highly specific gill parasites that has radiated elsewhere in Africa. Infection profiles of species of *Cichlidogyrus* differed between sympatric cichlid species only in the oldest and most differentiated pair, again inconsistent with parasite-mediated speciation. To conclude, parasites may contribute to host differentiation *after* speciation, but do not initiate host speciation.

Keywords: parasite-mediated selection, diversification, adaptive radiation, host–parasite interaction, Cichlidae, Monogenea

Introduction

Parasitism is a common lifestyle in nature, with all organismal groups being infected by parasites (Poulin, 1996). Parasites can affect host growth, reproduction, and survival (Agnew et al., 2000; Lafferty & Kuris, 2009; Segar et al., 2018); can manipulate host behavior (Poulin, 2010); impair host population growth (Hudson et al., 1998); and affect host fitness in general. Because of these effects, parasites are considered potential drivers of host evolution, including host ecological divergence and speciation (Kaltz & Shykoff, 1998). Ecological speciation occurs when reproductive isolation results from ecologically based divergent selection or from ecologically mediated divergent sexual selection (Nosil, 2012; Schluter, 2009), both potentially arising from heterogeneity in parasite infection among- or within-host populations (Karvonen & Seehausen, 2012). Even within a single population, individuals may encounter different parasites as a result of individual variation in microhabitat occupation and foraging strategies in a heterogeneous environment (Hablützel et al., 2017; Hayward et al., 2017). Unless resistance is achieved with a single polymorphic dominant allele, divergent adaptation in immunity traits within a population requires assortative mating, possibly mediated by mate choice targeting immunity traits. Parasite infection can affect mate choice, for example, through effects on condition-dependent expression of display traits (Hamilton & Zuk, 1982; Maan et al., 2008) or through MHC-mediated mate choice (Eizaguirre et al., 2011; Landry et al., 2001). Thus, parasites may initiate or contribute to speciation in their hosts (e.g., virus-induced allopatric divergence in *Pseudomonas* bacteria, Buckling & Rainey, 2002) or may strengthen differentiation after speciation, once reproductive isolation has already arisen by other mechanisms (Eizaguirre et al., 2009).

Cichlid fish of the African Great Lakes provide well-studied examples of adaptive radiation (Kocher, 2004; Kornfield & Smith, 2000; Seehausen, 2006), in which a large number of species have arisen and become ecologically diversified (Turner, 2007). Currently, African cichlid species display a large variation in morphology (e.g., body shapes, color patterns), behaviors, and trophic and microhabitat specializations (Fryer & Iles, 1972; Kornfield & Smith, 2000). Their diversity in the use of ecological niches suggests that different species may be exposed to different parasites. Indeed, divergent parasite infections have been observed among

cichlids in several of the African Great Lakes. Divergent parasite infections were found in Lake Tanganyika cichlids among allopatric color morphs of *Tropheus moorii* Boulenger, 1898 (Raeymaekers et al., 2013), among allopatric populations of two tropheine species (*Tropheus moorii*, *Simochromis diagramma* (Günther, 1894); Grégoir et al., 2015; Hablützel et al., 2016), and among several sympatric tropheine species (Vanhove et al., 2015). In Lake Malawi, differentiation in parasite community composition between two sympatric cichlid species was found to be correlated with differentiation in MHC genes (Blais et al., 2007). In Lake Victoria, where cichlid radiation has happened exceptionally rapidly (McGee et al., 2020), differences in parasite infections were found between several species in a sympatric cichlid species assemblage, and several of these differences were consistent over time (Gobbin et al., 2020; Karvonen et al., 2018).

Here, to establish whether parasites contribute to host speciation or whether they promote host differentiation after speciation, we investigate macroparasite infection in replicates of host species pairs that vary in the time since divergence and/or the extent of differentiation. Closely related sympatric species of *Pundamilia* (Cichlidae) co-occur in pairs at rocky islands in southeastern Lake Victoria (Figure 1A). The two sympatric species in each pair differ in their male nuptial coloration, bright red and yellow in one, blue-gray in the other. Between islands, the species pairs vary in the time since divergence, the extent of current reproductive isolation (Meier et al., 2017), and the extent of differentiation in male nuptial coloration (Seehausen et al., 2008), water depth occupation (Seehausen, 1996), morphological traits (van Rijssel et al., 2018), and opsin gene allele frequencies and opsin gene expression (Carleton et al., 2005; Seehausen et al., 2008; Wright et al., 2019). They differ in the extent of genetic differentiation, based on microsatellites (Seehausen et al., 2008) and SNPs (Meier et al., 2017, 2018). These parallel patterns of divergence in several traits suggest that similar mechanisms may be acting in multiple populations of *Pundamilia*. Previous studies have characterized the macroparasite infection in a strongly differentiated and reproductively isolated

sympatric pair of *Pundamilia* (Karvonen et al., 2018; Maan et al., 2008), documenting differences in macroparasite infection consistent with their differences in diet and microhabitat utilization.

In the present study, we characterize ecto- and endoparasite infections in four sympatric pairs of *Pundamilia*, varying in their age and extent of differentiation. If parasites initiate host speciation in *Pundamilia*, differentiation in parasite infection between sympatric blue and red populations should be present already before reproductive isolation becomes significant—rather than emerging only after speciation has occurred. In this scenario, we expect infection differentiation to increase faster than host neutral genetic differentiation (Figure 1B). If parasites contribute to, but do not initiate host speciation, we expect parasite-mediated selection to be strong enough to cause divergent adaptation in the host populations. In this scenario, infection differentiation is linearly correlated with genetic differentiation. If parasites do not play a role in host speciation, we expect infection differentiation to increase more slowly than genetic differentiation (i.e., host genetic changes are subsequently reflected by differentiation in infection) or to be unrelated to the latter. Finally, temporal consistency in parasite-mediated divergent selection is required for it to drive evolutionary divergence of host populations (Karvonen & Seehausen, 2012). Hence, we assessed the stability of infection differences between years.

Methods

Study system

The blue *Pundamilia pundamilia* (Seehausen et al., 1998) and the red *Pundamilia nyererei* (Witte-Maas & Witte, 1985) are two morphologically very similar cichlid species that co-occur at rocky islands in southeastern Lake Victoria. At Makobe Island (Speke Gulf), water transparency is relatively high and reproductive isolation between *P. pundamilia* and *P. nyererei* is complete, the species are at least 12,000 years old (Meier et al., 2017) and interspecific differentiation is high for several traits (Figure 1A; Seehausen et al., 1997). *Pundamilia*
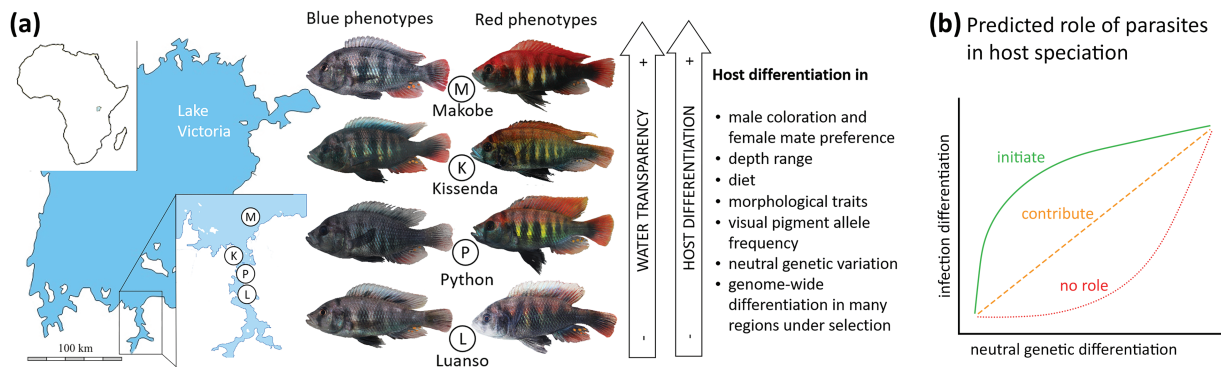
Figure 1. (A) Geographic location of sampled populations of *Pundamilia* in southeastern Lake Victoria, Tanzania. At Makobe Island (M), the blue *P. pundamilia* and red *P. nyererei* are strongly differentiated in several traits and reproductive isolation is complete. Populations inhabiting Python and Kissenda Islands have recently been shown to represent a separate speciation event; therefore, they are referred to as blue *P.* sp. "pundamilialike" and red *P.* sp. "nyererei-like." They show intermediate levels of genetic and phenotypic differentiation. At Luanso Island (L), male coloration ranges from blue to reddish with most males of intermediate coloration. There is no detectable genetic differentiation between the color morphs and we refer to them as *P.* sp. "Luanso." (B) Possible role of parasites in host speciation. Parasite-driven speciation would be supported if infection differentiation precedes host genetic differentiation (solid line). If parasites are an important component in driving host genetic differentiation (e.g., host adaptation to the parasite), then infection divergence and host genetic differentiation are positively correlated (dashed line). If parasites do not have a role in host speciation, infection differentiation arises after host genetic differentiation (dotted line) or it is unrelated to the latter.

pundamilia colonized the more turbid northern and central Mwanza Gulf (e.g., Kissenda, Python, and Luanso Islands) several thousand years ago and more recently received substantial gene flow from *P. nyererei* (Meier et al., 2017, 2018). At Python and Kissenda Islands, these hybrid populations subsequently speciated into sympatric species pairs with blue and red male nuptial coloration, resembling the original species (referred to as *P.* sp. "pundamilia-like" and *P.* sp. "nyererei-like," respectively). There, the two species show low levels of hybridization and intermediate levels of phenotypic and genetic differentiation (Maan & Sefc, 2013; Meier et al., 2017, 2018; Seehausen & van Alphen, 1998; Selz et al., 2014). At Luanso Island, where the water is turbid, there is a single panmictic and genetically undifferentiated population of *Pundamilia* (*P.* sp. "Luanso"). It is considered a single species with blue, intermediate and red male color morphs. From now on, we use "form" to denote the blue or red male color morphs, "island" to refer to sampling locations and "population" for island-form combinations.

In Lake Victoria, cichlids are infected by gill parasitic monogenean flatworms (Platyhelminthes: Monogenea), copepods (Crustacea: Copepoda), bivalves (Mollusca: Bivalvia), and by endoparasitic nematodes and trematodes (Gobbin et al., 2020; Karvonen et al., 2018; Maan et al., 2008). Copepod species infecting cichlids have a direct life cycle, usually with only the adult female attaching to the gills (Boxshall & Halsey, 2004). Bivalves encyst in fish gills only at larval stage (i.e., glochidia; Modesto et al., 2018). Both trematodes and parasitic nematodes have an indirect life cycle, with cichlids usually being intermediate hosts to nematode larvae (Scholz et al., 2018). Host specificity of these four parasite groups is generally low in Lake Victoria cichlids (Gobbin et al., 2020; Scholz et al., 2018). We focus on gill parasitic monogenean flatworms, which are often used as model systems for studying the evolutionary history of host–parasite interactions (Boeger & Kritsky, 1997; Brooks, 1979; Cruz-Laufer et al., 2021, 2022). One dactylogyridean flatworm genus in particular, *Cichlidogyrus*, is a good candidate for driving host diversification in cichlids because of its direct life cycle, high host specificity, large species number, and because it has undergone at least one lacustrine radiation, in Lake Tanganyika (Pariselle et al., 2003; Vanhove et al., 2015, 2016). Attachment to the gills causes secretion of mucus, hyperplasia, and infiltration of neutrophils (Igeh & Avenant-Oldewage, 2020). Therefore, in addition to parasitological analysis where species of *Cichlidogyrus* are pooled, we also analyze the species communities of *Cichlidogyrus*. From now on, "ecto-endoparasite community" refers to the assemblage of ecto- and endoparasite taxa (pooling species of *Cichlidogyrus* as one taxon, and including all other parasite taxa recovered), whereas "*Cichlidogyrus* community" refers to the assemblage of species of *Cichlidogyrus*.

Fish collection

Fish were collected in May–August 2010 and June–October 2014 at four locations in southeastern Lake Victoria: *P. pundamilia* and *P. nyererei* at Makobe (−2.3654, 32.9228); *P.* sp. "pundamilia-like" and *P.* sp. "nyererei-like" at Kissenda (−2.5494, 32.8276) and Python Islands (−2.6238, 32.8566) and *P.* sp. "Luanso" at Luanso Island (−2.6889, 32.8842; Figure 1A). Collection was done by angling or with gillnets of variable mesh sizes, set at different depths (0.5–18.5 m). Because of the difficulty to identify females in the field, and

to avoid confounding species differences with differences between the sexes (Maan et al., 2006; Zuk & McKean, 1996), only males were investigated. Fish at Luanso were categorized into blue or red morphs (or intermediate, in 2014 only) by visual scoring of male nuptial coloration by multiple observers (following Seehausen et al., 2008). Fish were tranquilized and subsequently sacrificed with an overdose of 2-phenoxyethanol (2.5 mL/L). Some fish were preserved in 4% formalin and subsequently transferred to 70% ethanol; other fish were directly preserved in 70% ethanol to allow future genetic studies. Before preservation, the body cavity of the dead fish was slit open ventrally to allow preservation of internal organs and parasites. Each individual fish was measured (standard length and body depth, to the nearest 0.1 mm) and weighed (to the nearest 0.1 g; Supplementary Table S1).

Parasite screening and identification of species of *Cichlidogyrus*

Under a dissecting stereoscope, we examined gill arches (right side of the fish only) for ectoparasites and abdominal cavity, gonads, liver, and gastrointestinal tract for endoparasites. All macroparasites were identified following Paperna (1996).

All *Cichlidogyrus* collected in 2014 were individually preserved in 100% ethanol. For morphological identification, we selected a subset of specimens of *Cichlidogyrus* (*n* = 193) from the host populations at three of the four study sites: Makobe, Kissenda, and Luanso (Python was excluded because it is very similar to Kissenda). We sampled all individuals of *Cichlidogyrus* infesting each individual host, until a minimum of 24 specimens of *Cichlidogyrus* per host population was obtained.

For morphological species identification, specimens of *Cichlidogyrus* were mounted on slides in Hoyer's medium, after prior treatment with 20% sodium dodecyl sulphate to soften tissues. Specimens of *Cichlidogyrus* were examined with a microscope (Olympus BX41TF) under 1,000× magnification using differential interference phase contrast. Although most of the species of *Cichlidogyrus* that we found in Lake Victoria cichlids are not formally described, species could be discriminated based on the shape and size of the sclerotized parts of the attachment organ (haptor) and, in particular, of the male copulatory organ (MCO; e.g., Gobbin et al., 2020; Grégoir et al., 2015).

For each parasite taxon and host population, we calculated (a) infection prevalence, that is, proportion of infected individuals in each host population; (b) infection intensity, that is, number of parasites per infected host individual (excluding uninfected individuals); and (c) abundance, that is, number of parasites per host individual, both infected and uninfected.

Data analysis

Infection divergence among host populations

To investigate whether the composition of ecto-endoparasite communities differed between sympatric forms of *Pundamilia*, we performed analysis of similarities based on zero-adjusted Bray–Curtis similarity indices (Clarke et al., 2006) of parasite abundance (ANOSIM, 9999 permutations, PAST software, Hammer et al., 2001). The two sampling years were analyzed separately. Pairwise comparisons were made using the false discovery rate correction for *p* values (Benjamini & Hochberg, 1995). We evaluated the effect of differences in
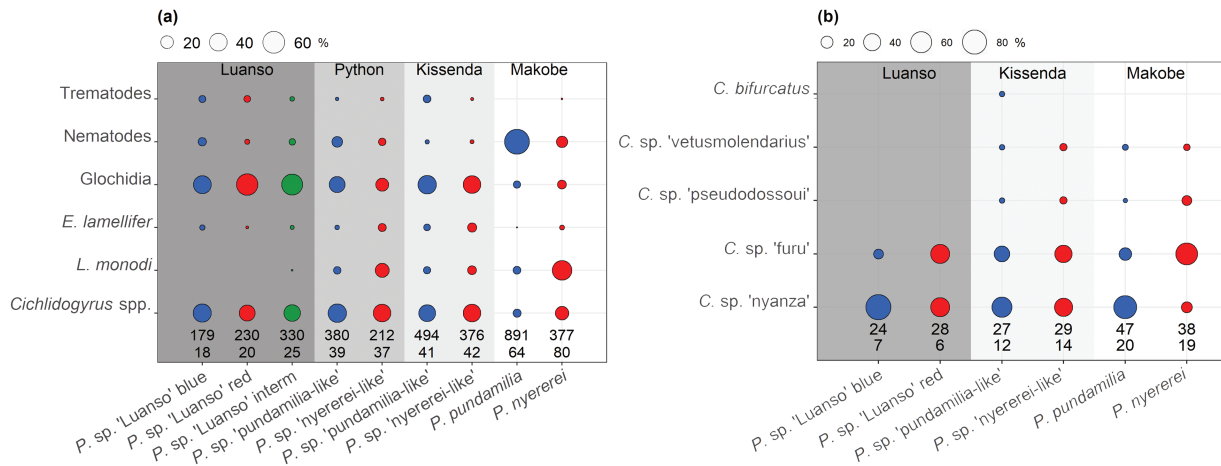
Figure 2. Infection profiles of forms of *Pundamilia* with (A) ecto-endoparasites (both sampling years) and (B) species of *Cichlidogyrus* (only 2014 available) at the sampled localities with different extents of genetic differentiation between host forms. Infection profiles differed between sympatric blue and red males of *Pundamilia* only at Makobe, where these represent relatively old species, reproductive isolation between them is complete and ecological and genomic differentiation is strongest. Bubble size represents the proportion of individuals belonging to a specific parasite taxon, relative to the total number of individuals of such parasite taxon in a host population. Numbers refer to total sample sizes of hosts and parasites.

host diet and water depth occupation on infection variation using PERMANOVA (PAST, see Supplementary Material). Differences in infection parameters (prevalence, intensity, abundance) between host populations were also analyzed for each parasite taxon separately using generalized linear models in R (R Core Team, 2019, Supplementary Material). Fish body length was not included in the models because interspecific differences in body length were consistent across the four islands (Supplementary Figure S1), while correlations between body length and infection abundance were inconsistent across host populations (Supplementary Figure S2). Hence, body size variation between host individuals does not explain infection differences between host populations.

To assess whether parasite community dissimilarity covaried with the extent of genetic differentiation and/or the geographic distance between populations, we first constructed matrices of pairwise host population differences in ecto-endoparasite community dissimilarity (based on zero-adjusted Bray–Curtis distances, SIMPER, PAST), F_{ST} distances (data taken from Meier et al., 2017), geographic distances, and male phenotypic differentiation (assigning a value of 0 to same and 1 to different male color forms). The two sampling years were analyzed separately. The relationship between ecto-endoparasite community dissimilarity and host genetic differentiation was analyzed with a Spearman Mantel test (9,999 permutations), using residuals from the regression of parasite community dissimilarity and of genetic distance on geographic distance to control for the effect of geographic distance on both other distances. We used the same method to assess the relationship between geographic distance and ecto-endoparasite community dissimilarity (controlling for host genetic distance) and the relationship between phenotypic differentiation and ecto-endoparasite community dissimilarity (controlling for geographic distance and genetic distance separately; Supplementary Material). Also for the individual parasite taxa in the ecto-endoparasite community, we explored whether the difference in abundance between host populations increased with increasing genetic differentiation between them or with increasing geographic distance (Supplementary Material). The same statistical procedures were applied to investigate variation in communities of

Cichlidogyrus, in abundance of each *Cichlidogyrus* species separately and to correlate the infection differentiation in *Cichlidogyrus* to host genetic differentiation.

Temporal consistency of infection

To investigate temporal consistency in infection, we compared ecto-endoparasite community composition between samples collected in 2014 and 2010 (ectoparasite data for 2010 were from Karvonen et al., 2018), using ANOSIM based on zero-adjusted Bray–Curtis distances of parasite abundance (9,999 permutations, PAST). Temporal consistency of infection was also assessed using generalized linear models for each ecto- and endoparasite taxon (pooling species of *Cichlidogyrus*) separately, but not for *Cichlidogyrus* species as they were identified in 2014 only (Supplementary Material).

Results

In total, we sampled 368 male individuals (163 blue, 180 red, and 25 intermediate color forms; Supplementary Table S1). We observed four ectoparasite taxa and two endoparasite taxa (Figure 2A, Supplementary Figure S3, Supplementary Table S2). Ectoparasites were as follows: *Cichlidogyrus* spp. (Monogenea: Dactylogyridea), *Lamproglena monodi* Capart, 1944 (Copepoda: Cyclopoida), *Ergasilus lamellifer* Fryer, 1961 (Copepoda: Poecilostomatoida), and glochidia larvae of mussels (Bivalvia: Unionoidea). Endoparasites were nematodes and trematodes (not further identified). The most common parasite was *Cichlidogyrus* (overall prevalence 68%).

Morphological assessment of *Cichlidogyrus* revealed five different species (Figure 2B, Supplementary Table S3): *C. bifurcatus* Paperna, 1960, *C.* sp. "furu," *C.* sp. "nyanza," *C.* sp. "pseudodossoui," *C.* sp. "vetusmolendarius" (Gobbin et al., 2020; 2021a, 2021b).

Infection differences between host populations

Consistent with the first and second requirements for parasite-mediated speciation (Karvonen & Seehausen, 2012), all host populations (both sympatric and allopatric) differed in the communities of ecto- and endoparasites in both
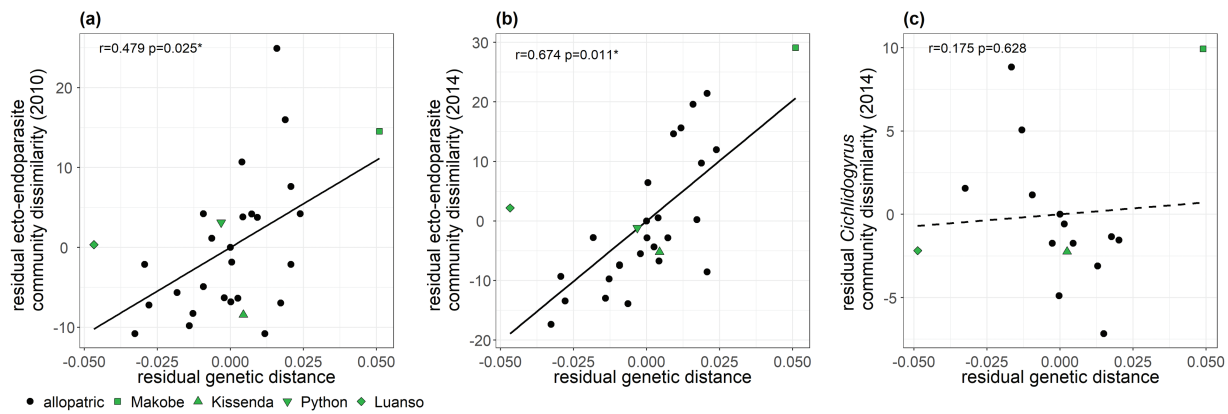
Figure 3. Correlation between the genetic distance between populations of *Pundamilia* and the dissimilarity of their ecto-endoparasite communities (F_{ST} values based on RADseq SNPs from Meier et al., 2017), controlling for geographic distance, (A) in 2010, (B) in 2014, (C) considering species of *Cichlidogyrus* in 2014. Infection dissimilarities and genetic distances are plotted as residuals from separate regressions against geographic distance. Symbols other than points indicate sympatric pairs of blue and red forms.

years (2014: $p < .0001$, $R_{ANOSIM} = 0.409$, $n = 83$; 2010: $p < .0001$, $R_{ANOSIM} = 0.373$, $n = 75$) and in the communities of *Cichlidogyrus* species (available for 2014 only; $p = .0039$, $R_{ANOSIM} = 0.098$, $n = 79$).

However, these differences in parasite communities were largely driven by the Makobe populations, where host species are highly differentiated and reproductively isolated (ecto-endoparasite community 2010: $p = .004$, $R_{ANOSIM} = 0.429$, $n = 74$; ecto-endoparasite community 2014: $p < .0004$, $R_{ANOSIM} = 0.862$; $n = 82$; *Cichlidogyrus* community 2014: $p < .008$, $R_{ANOSIM} = 0.242$, $n = 78$; Supplementary Table S4, Figure 2). The other sympatric *Pundamilia* forms did not differ in ecto-endoparasite community (2010: $p > .215$; 2014: $p > .115$) or *Cichlidogyrus* community ($p > .329$; Supplementary Table S4). The only exception was Python Island in 2010, where blue and red forms differed in ecto-endoparasite community ($p = .004$; $R_{ANOSIM} = 0.293$, $n = 19$; but not in 2014). Variation in the magnitude of infection dissimilarity supported the observed pattern: the extent of infection differences between sympatric blue and red forms was stronger at Makobe than at other islands, for ecto-endoparasite communities in both years and for the *Cichlidogyrus* community in 2014 (Supplementary Table S4).

Temporal consistency in infection

Parasite community composition was similar between years (2014 vs. 2010 $p = .807$, $R_{ANOSIM} = -0.021$, $n = 156$). In both years, sympatric blue and red hosts differed in parasite community composition at Makobe (*P. pundamilia* had more nematodes, *P. nyererei* had more copepods, that is, *L. monodi* and *E. lamellifer*), but not at other islands (Supplementary Figure S3). An exception is Python Island, where the red species had more copepods than the blue species in 2010, but not in 2014.

For all cases in which sympatric host forms differed significantly in infection abundance in both years ($n = 4$), or tended to differ ($p < .1$; $n = 1$), the direction of the difference was maintained between years (Supplementary Figure S4).

Infection differentiation covaries with host genetic differentiation

The scenario of parasites contributing to host speciation (but not initiating it) was supported by the positive linear correlation between parasite community dissimilarity among host populations and host genetic differentiation, in both sampling years (both sympatric and allopatric pairs, after geographical distance was accounted for, Figure 3A and B; Supplementary Table S5). This linear pattern was supported by comparing the Akaike information criterion for alternative model fits (AIC 2010: linear 270.19, quadratic 270.31, exponential 307.47; AIC 2014: linear 303.82, quadratic 307.47, exponential 469.87). This positive relationship was driven by the blue–red sympatric comparison at Makobe island, and it was also observed when only considering allopatric forms (Supplementary Figure S5). Ecto-endoparasite community dissimilarity did not increase with geographic distance, after genetic differentiation was accounted for (Supplementary Figure S6; Supplementary Table S5, Supplementary Material).

The dissimilarity in the community of *Cichlidogyrus* species among host populations (sympatric and allopatric pairs) was not associated with host genetic differentiation or with geographic distance (controlling for geographic or genetic distance, respectively; Figure 3C, Supplementary Figure S6c, and Supplementary Table S5).

Discussion

Parasites can have strong eco-evolutionary interactions with their hosts, and therefore, they may have a role in host speciation (Haldane, 1949; Kaltz & Shykoff, 1998; Karvonen & Seehausen, 2012). Although studies on parasite-mediated speciation have begun to accumulate (Eizaguirre et al., 2011, 2012; Feulner et al., 2015; Greischar & Koskella, 2007; Karvonen et al., 2015; Stutz et al., 2014), it is still unclear at what stage of the speciation process parasite-mediated divergent selection acts, especially in the context of adaptive radiation (El Nagar & MacColl, 2016; Vanhove & Huyse, 2015). To investigate the role of parasites in host speciation we documented patterns of ecto- and endoparasite infection in sympatric and allopatric populations of *Pundamilia* cichlids in four communities in Lake Victoria. We found that differences in parasite communities increased with the genetic differentiation between host populations, but only the most differentiated host pair differed significantly in infection. This pattern was observed in two sampling years (Figure 3A and B).

Infection differences arise after speciation

The linear relationship between differentiation in ecto-endoparasite infection and host genetic differentiation suggests that infection differences between cichlid host species accumulate with time after the emergence of isolation (geographical, behavioral, or both) alongside increasing host genetic divergence, rather than preceding it. This supports a scenario in which parasites contribute to, but do not initiate, host population divergence. Although we cannot exclude that divergence in infection arose as a by-product of host genetic differentiation, we may speculate that this process would take time and thus generate a concave relationship between infection differentiation and host genetic differentiation, rather than a linear one as observed here (Figure 1B). Consistent with our results, Karvonen et al. (2018) found a positive correlation between infection dissimilarity and host genetic distance in ectoparasites of the same *Pundamilia* species pairs. Differences in infection abundance were positively correlated with genetic distance also in stickleback allopatric stream populations and parapatric lake-stream ecotype pairs (Karvonen et al., 2015). On the other hand, no association between infection differences and host genetic distance was observed among allopatric color morphs of *Tropheus* cichlids in southern Lake Tanganyika, that diverged much longer ago than *Pundamilia* (18–250 KYA; Raeymaekers et al., 2013). This may be due to a ceiling effect of the divergence time and/or the weaker ecological differentiation of the *Tropheus* morphs compared with the *Pundamilia* morphs.

The scenario of parasites contributing to but not initiating divergence is also supported when considering only sympatric host populations. Among the sympatric pairs, infection differences (in ecto-endoparasite community, in *Cichlidogyrus* community, and in the abundances of *L. monodi*, nematodes and *Cichlidogyrus* sp. "nyanza") were largely driven by one of the four sampled species pairs, from Makobe Island, where the blue and red host species are relatively old and genetically strongly differentiated (Meier et al., 2017, 2018). Again, this suggests that parasites do not initiate speciation in *Pundamilia* cichlids, but may contribute to host differentiation after speciation (or geographical isolation) has already occurred. However, we saw some indications of differences in infection also between young sympatric host species (abundance of trematodes at Kissenda and abundance of *Cichlidogyrus* spp. at Python), suggesting that infection differences can arise early after or during speciation. At Luanso, where both male nuptial coloration and female mating preferences for male coloration vary within the population (van der Sluijs et al., 2007; Seehausen et al., 2008), even though the population appears panmictic at genomic SNPs, we did not observe infection differences between morphs. Heterogeneous parasite infection is hence unlikely to be a driver of the differentiation in male nuptial color and associated female mating preferences.

Cichlidogyrus spp. are gill parasites that are considered good candidates for driving parasite-mediated speciation because its members are often highly host specific and have radiated in at least one other African lake. In our study however, infection dissimilarity in terms of *Cichlidogyrus* species community was not associated with host genetic differentiation. Among sympatric pairs, only the genetically highly differentiated species pair at Makobe Island differed in *Cichlidogyrus* community and in the abundance of one species of *Cichlidogyrus*. This suggests that divergent *Cichlidogyrus* infection profiles do not emerge until quite long after speciation when host species are strongly genetically differentiated. The host species pair at Makobe Island is about 15,000 years old, whereas those at Python and Kissenda Islands are perhaps less than 1,000 years old (Meier et al., 2017). This is in line with our earlier study in a cichlid assemblage of 16 sympatric species at the same location (Makobe Island). We observed that the *Cichlidogyrus* species community did not differ among the species of the Lake Victoria radiation, but did differ between these and two distantly related cichlid species (*Astatoreochromis alluaudi*, *Pseudocrenilabrus multicolor*) belonging to lineages that have not speciated in Lake Victoria (Gobbin et al., 2020). The lack of infection differentiation at *Cichlidogyrus* species level among recently diverged host species may represent an example of ecological fitting via resource tracking (Agosta & Klemens, 2008). Namely, young host species may represent sufficiently similar resources for *Cichlidogyrus*, such that individual parasite species can perform equally well on all of them. This may imply that *Cichlidogyrus* may have only minor fitness effects for the host and thereby exert only weak selection for species-specific defense mechanisms (tolerance or resistance).

The extent of parasite community dissimilarity between all host populations (sympatric and allopatric) was unrelated to geographic distance between them (after genetic differentiation was accounted for) in both sampling years. These findings suggest that the host infection profile is primarily determined by host genetic differentiation (which can be caused by geographical isolation or by ecological differentiation) rather than by geographic distance only. Infection variation was not correlated with geographical distance in Tropheini in Lake Tanganyika (Hablützel et al., 2016).

Temporal consistency of infection differences

The direction of parasite-mediated selection must be consistent over time for adaptive divergence to occur (Karvonen & Seehausen, 2012). Such temporal stability in differences between parasite assemblages was previously observed in cichlids of Lake Tanganyika (Raeymaekers et al., 2013) and Lake Victoria (Gobbin et al., 2020). In the present study, ecto-endoparasite community composition was similar in both sampling years, and in both years, blue and red species differed most strongly at Makobe.

When considering each parasite taxon separately (but pooling species of *Cichlidogyrus*), sympatric host species that differed significantly in infection abundance, differed in the same direction in both sampling years (5 of 5 cases, Supplementary Figure S4, Supplementary Material). Thus, the direction of blue–red infection differences was maintained, despite year-to-year variation in parasite abundances (and hence presumably in selection strength). Previous studies, on samples collected in 2003 at Makobe and in 2005 at Kissenda, reported the same direction of infection differences as observed in the present study, for nematodes and copepods (Desêtres, 2010; Maan et al., 2008). Thus, we conclude that the infection differences between sympatric cichlid species are consistent over time.

Conclusion

We found differences in parasite infection among blue and red *Pundamilia* populations in Lake Victoria, and these were largely consistent over several sampling years.

When looking at host species pairs of different ages (either sympatric or allopatric), we found that species differences in infection accumulate only after speciation, suggesting that parasites may contribute to strengthening differentiation between ecologically divergent host species, rather than initiating host speciation. Parasite assemblages were relatively similar among allopatric host populations with weak genetic differentiation and became more divergent as genetic differentiation among host populations increased. This is consistent with a gradual accumulation of infection differences among reproductively isolated host populations, and is also consistent with parasite-mediated divergence in allopatry.

To conclude, our study is consistent with a contribution of parasites to divergent evolution between host species, but does not support parasite-initiated speciation in the Lake Victoria cichlid radiation.

Supplementary material

Supplementary material is available online at *Evolution* (https://academic.oup.com/evolut/qpad080).

Data availability

The data underlying this article are available in Dryad Digital Repository at https://doi.org/10.5061/dryad.c866t1gbh.

Author contributions

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