The role of parasites in host speciation

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English summary

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PARASITE-MEDIATED SPECIATION

Speciation – the formation of new species – was defined by Darwin as the "mystery of mysteries" more than a century ago. Since then, mechanisms of speciation have been investigated intensively and progress has been made. Adaptations to biotic and abiotic factors may cause speciation as a by-product, and parasites may be an important biotic agent of selection. However, some mechanisms remain under-explored. In particular, the onset of divergence is still not well understood. In this thesis, I investigate when and how parasite-mediated divergent selection contributes to speciation process.

Parasites constitute a widespread source of ecological selection (Poulin & Morand, 2000; Schmid-Hempel, 2013), that may potentially act as a driver of speciation (Schluter, 1996, 2000b; Rundle & Nosil, 2005; Maan & Seehausen, 2011). By definition, parasites impose fitness costs on their hosts (e.g. reduced growth, reproduction and survival, Agnew et al., 2000; Lafferty & Kuris, 2009; Segar et al., 2018). Hosts adapt to parasites by evolving resistance, tolerance or behavioural avoidance. In turn, parasites counter-adapt by evading or suppressing host immunity. This leads to a coevolutionary dynamic of adaptation and counter-adaptation (Decaestecker et al., 2007).

Host populations occupying different ecological niches may be exposed to different parasite numbers and species, potentially resulting in different parasite-mediated selection (Knudsen et al., 2004; Pegg et al., 2015; Hablützel et al., 2017; Hayward et al., 2017) even in sympatry. This may lead host populations to evolve different adaptations against local parasite threats. Such adaptive responses can lead to an increasingly different parasite infection pattern between host populations. If these differences are maintained over time, then parasite-mediated selection continuously acts in the same direction, promoting host divergence. Stochastic or frequency-dependent temporal fluctuations in parasite abundances could cause variation in the strength of parasite-mediated selection, but divergence is promoted as long as the direction of selection is maintained (**Fig. 9.1**).

Such divergent and temporally stable differences in infection may lead to genetic differentiation between host populations, and eventually drive or strengthen reproductive isolation between them (Hamilton & Zuk, 1982; Landry et al., 2001; Nosil et al., 2005; Maan et al., 2008; Eizaguirre et al., 2011). Moreover, reproductive isolation could be reinforced by selection against hybrids and immigrants (i.e. with higher infection), immune-mediated mate choice (i.e. choice for partners providing locally adaptive immunity) or parasite-mediated mate choice (i.e. choice for healthy partners). Alternatively, parasite-mediated divergent selection may strengthen host differentiation once a certain level of reproductive isolation is already achieved through other mechanisms (Haldane, 1949; Price et al., 1986; Karvonen & Seehausen, 2012).

The study of parasite-mediated speciation can be problematic because of the two-way nature of the host-parasite interaction – one needs to determine which of the two is driving the diversification – and because of the involvement of other ecological factors – one needs to distinguish the effects of parasites from those of other potential drivers of diversification. To this end, host populations at early stages of speciation provide a good model system. In this thesis, I take advantage of the young adaptive radiation of cichlid fish in Lake Victoria to investigate the role of parasites in host speciation.

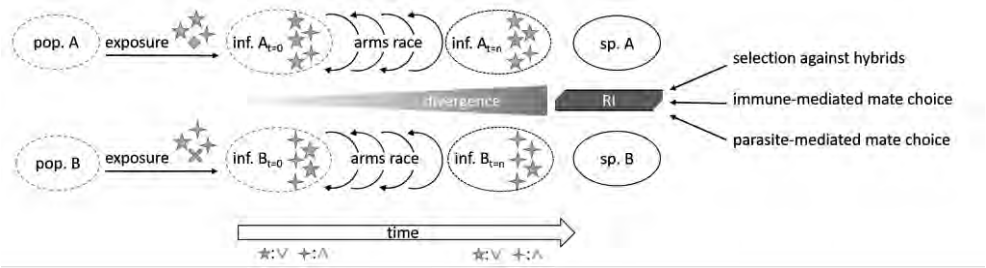

Figure 9.1
Parasite-mediated speciation. Two interbreeding host populations occupy two different ecological niches (A and B) and are exposed to different numbers and species of parasites (symbols), resulting in two different infection patterns. Each host population evolves adaptations against local parasites, engaging in an evolutionary arms race. The direction of infection differences remains consistent over time despite fluctuations in overall abundance (★ higher in population A than in B, ✦ lower in A than in B). Divergence in defences against parasites leads to reproductive isolation (RI) between host populations – that may be reinforced by selection against hybrids and/or immigrants, and/or immune-mediated and/or parasite-mediated mate choice– resulting in two distinct host species.

LAKE VICTORIA CICHLIDS AND THEIR PARASITES

The adaptive radiation of cichlid fish in Lake Victoria is particularly suitable for studying parasite-mediated speciation, because of its young age, interspecific ecological diversity and relatively weak genetic differentiation. As recently as 14'600 years ago, the lake refilled after being dry for thousands of years (Johnson et al., 1996; Stager & Johnson, 2008). A hybrid swarm formed after colonization of the refilled lake by two riverine lineages (Seehausen et al., 2003; Meier et al., 2017a), providing the genetic variation that facilitated the rapid adaptive speciation (Seehausen, 2004; Salzburger, 2018). Thus, most Victorian cichlids evolved *in situ* after that dry period (Johnson et al., 2000; Stager & Johnson, 2008; Wagner et al., 2013; Meier et al., 2017a). In Lake Victoria, radiation members co-occur with old and distantly related lineages that did not speciate after colonizing the lake: *Astatoreochromis alluaudi* (Pellegrin, 1904), *Pseudocrenilabrus*

multicolor (Schöller, 1903), *Oreochromis variabilis* (Boulenger, 1906) and *Oreochromis esculentus* (Graham, 1928). These provide a helpful comparison to study why some lineages speciated and others did not.

Previous studies suggest that parasite-mediated speciation might happen in cichlids, because of strong diversity in ecological niches (Fryer & Iles, 1972; Wagner et al., 2012a), high potential for disease transmission because of high fish densities (Ribbink et al., 1983; Fenton et al., 2002), association between parasitism and the expression of sexual signals (Taylor et al., 1998; Maan et al., 2006b) and rapid evolution of MHC genes (Blais et al., 2007). Moreover, there is evidence for co-evolution between cichlids of Lake Tanganyika and their monogenean gill parasites (Vanhove et al., 2015).

Lake Victoria also harbours replicates of speciation at different stages, which allows to assess when – during the speciation process – differences in infection arise. The blue *Pundamilia pundamilia* (Seehausen et al., 1998) and the red *Pundamilia nyererei* (Witte-Maas and Witte, 1985) are two closely related cichlids that co-occur at rocky islands in the southeastern part of the lake. At some locations, these two species hybridized and then speciated again into similar blue and red pairs (Meier et al., 2017b; Meier et al., 2018). Across locations, blue and red forms vary in the extent of genetic differentiation (Seehausen et al., 2008; Meier et al., 2017b; Meier et al., 2018), morphological differentiation (van Rijssel et al., 2018a), differentiation in visual adaptation (Carleton et al., 2005; Seehausen et al., 2008; Wright et al., 2019), and in the frequency of hybridisation (Seehausen, 1996a; Seehausen et al., 2008; Meier et al., 2017b; Meier et al., 2018). Variation in these traits is associated with water transparency.

Parasites live at the expenses of their hosts, thereby imposing a fitness cost. The parasite life cycle can entail one or more host species (the final host harbouring reproductive adults) and may also include free-living stages (often eggs or larvae). Cichlids are hosts to many macroparasite taxa: monogeneans (gill parasites, but some genera infect digestive tract or bladder, with a direct life cycle), copepods (gill or skin parasites, with a direct life cycle), bivalve molluscs (gill or skin parasites, with a direct life cycle), nematodes (endoparasites, often fish are intermediate hosts), trematodes (flukes, endoparasites with at least two intermediate hosts). Monogeneans are of great interest in the study of host-parasite interactions because of their high host specificity. In particular, the gill parasite *Cichlidogyrus* is a good candidate for promoting host speciation, because of its high number of species, that differ in morphology, they display a high host specificity (Pariselle et al., 2003; Vanhove & Huyse, 2015; Vanhove et al., 2015).

THIS THESIS

In this thesis, I investigate whether parasites drive or contribute to host speciation in cichlid fish of Lake Victoria. To this end, I analysed the macroparasite infection of a large sympatric community of 17 cichlid species that radiated and two species representing lineages that never speciated (**chapters 2 and 5**), as well as infection differences between four replicates of blue and red *Pundamilia* pairs that vary in the extent of genetic differentiation (**chapters 3 and 4**). Fish were infected by five genera of gill parasites (*Cichlidogyrus* spp., *Gyrodactylus sturmbaueri*, *Lamproglena monodi*, *Ergasilus lamellifer*, glochidia larvae of bivalves) and two endoparasites in the abdominal cavity (nematodes, trematodes). Despite being good candidate for promoting cichlid speciation, *Cichlidogyrus* of Lake Victoria are mostly unknown. Thus, I morphologically identified them to species level. Infection patterns were analysed at between parasite genera level for two sampling years (2010 and 2014) and at within-*Cichlidogyrus* level for one sampling year (2014). Since results differed according to the parasite level analysed, I present them separately.

Infection differences at parasite higher taxon level

In **chapters 2 and 3** I tested two prerequisites for parasite-mediated speciation: *i)* species differences in infection and *ii)* temporal consistency in the direction of parasite-mediated selection (Karvonen & Seehausen, 2012). Seventeen sympatric host species occurring at Makobe and four pairs of blue and red forms of *Pundamilia* at four locations differed in their parasite infection, both in terms of parasite abundance and diversity, consistent with divergent parasite-mediated selection. These infection differences were mostly consistent between the two sampling years within all sampled species of the radiation and within the reproductively isolated sister species, supporting the temporal consistency hypothesis for parasite-mediated speciation.

In **chapter 3**, I assessed whether infection differences between species of blue and red *Pundamilia*, from four locations, covary with the extent of genetic or geographic distance between them. Only the most genetically differentiated blue-red sympatric pair differed in infection profiles. Comparison of all species pairs (sympatric and allopatric, of same and different colour) revealed that the extent of parasite community dissimilarity increased with increasing genetic distance within pairs, taking into account geographic distance among islands. These results suggest that species differences in infection depend on the extent of host genetic differentiation: infection differences accumulate as host genetic divergence increases, rather than precede genetic divergence. Therefore, parasites may contribute to host species differentiation but do not drive it. The positive correlation between infection differentiation and genetic differentiation at parasite higher taxon level was observed in both sampling years, supporting the consistency of parasite-mediated selection over time.

Although the intensity of some parasites was associated with water depth, variation in infection among host species was not fully explained by water depth and trophic specialization (**chapters 2 and 3**). This suggests that other intrinsic species properties (i.e. immunity and genetic susceptibility) also play a role. In **chapter 4**, I investigated the contribution of host intrinsic properties to variation in infection, by assessing infection differences in laboratory conditions with uniform exposure. I compared infection patterns between wild caught and first-generation lab-reared hosts of one of the *Pundamilia* pairs of chapter 3, as well as lab-reared interspecific hybrids. Prevalence and abundance of three of the most common ectoparasites were similar between lab and field. The two species differed in infection in the wild but not in laboratory conditions, where fish cannot express some species-specific ecological traits (e.g. depth and diet preferences). This indicates that variation in infection is mainly due to extrinsic effects rather than genetically based species differences in immunity. Since this pair of *Pundamilia* is weakly genetically differentiated, it is unlikely that differences in immune traits evolved already at early stages of speciation, which is inconsistent with a parasite contribution to divergence of *Pundamilia*.

Hybrids did not differ in infection from either parental species (all lab-bred first-generation, **chapter 4**), inconsistent with a hybrid disadvantage that would promote parasite-mediated diversification. Despite this, hybrids are rare in the field, likely because of species-assortative mating. The lack of hybrid disadvantage suggests that assortative mating is driven by other ecological factors.

In the wild, because of depth segregation, the two species of *Pundamilia* are adapted to different visual environments: blue forms inhabit a broad-spectrum light environment, while red forms inhabit a red-shifted light environment. These two visual conditions were mimicked in the laboratory. A mismatch in the visual environment of the hosts coincides with lower survival (Maan et al., 2017) and may coincide with higher parasite infection. This was not observed: parasite infection did not differ between natural and unnatural light conditions, suggesting that a visual mismatch does not increase host susceptibility.

Infection differences at within-*Cichlidogyrus* level

Contrary to what found at parasite higher taxon level, the *Cichlidogyrus* species community composition was similar within the sampled species belonging to the Lake Victoria radiation (**chapter 2**). This does not support a role of *Cichlidogyrus* in host diversification, as recently diverged radiation members were expected to evolve species-specific resistance linked to infection divergence. Instead, community composition of species of *Cichlidogyrus* differed between the three host lineages – the radiation lineage and the two older lineages represented by *A. alluaudi* and *Ps. multicolor*. Despite full sympatry of the hosts, *Cichlidogyrus* species infecting one lineage rarely infected another lineage, suggesting an opportunity for host

specialisation (although radiation members currently do not represent different resources for species of *Cichlidogyrus*).

When focusing on all pairs of *Pundamilia*, there was no gradual increase in the extent of dissimilarity in the community of *Cichlidogyrus* with genetic differentiation between populations (**chapter 3**), contrary to what found at parasite higher taxon level. As observed at parasite higher taxon level, the only sympatric comparison where community composition of *Cichlidogyrus* differed was in the reproductively isolated blue-red pair at Makobe. This indicates that *Cichlidogyrus* is not driving differentiation in *Pundamilia*. Instead, this suggests that differences in infection arise when hosts have already achieved a certain extent of divergence, contrary to a role of *Cichlidogyrus* in the early stages of host diversification. Together, these results suggests that species of *Cichlidogyrus* do not contribute to host differentiation.

No geographical pattern in species infection differences

Chances of getting infected and the numbers of parasite infecting hosts varied between locations. This may be explained by ecological differences between locations. For example, the highest infection levels of nematodes (often transmitted by birds) were observed at Makobe island, where large populations of cormorants and egrets occur. Abundances of parasites were generally low at the swampy location with few fish species and individuals, compared to rocky islands with relatively large cichlid populations. Despite such geographical variation in infection levels, species differences in infection were consistent across locations (**chapters 2 and 3**). This pattern was observed for single parasite taxa (e.g. red forms of *Pundamilia* harboured consistently more *L. monodi* and *E. lamellifer* than the blue forms) but also at the parasite community composition level (an increase in geographical distance between populations did not coincide with an increase in parasite community dissimilarity). An absence of geographical pattern in sympatric species differences in infection may support a sympatric parasite-mediated scenario, as differentiation in infection may result from intrinsic host traits (including resistance).

Parasite microhabitat segregation

In **chapter 5**, I analysed the micro-habitat distribution of parasites on the gills, to assess whether this could constitute another axis of divergence in infection. The two most abundant ectoparasite taxa (*Cichlidogyrus* spp., *L. monodi*) and species of *Cichlidogyrus* (*C. nyanza*, *C. furu*) had non-random microhabitat distributions that differed between host species, suggesting that the same parasite may interact differently with different host species. This may provide opportunity for parasite-mediated host differentiation. Microhabitat selection represents another axis of infection heterogeneity that may reveal more differences than parasite counts, hence is worthy including in future studies. Parasite interspecific relationships did not differ between host species. In monogeneans it may be explained by increasing opportunities of

mating (as they reproduce on the host); whereas in copepods it may be explained by egg exposure to water flow (as most copepods are attached in a way that exposes egg clutches outside gill filaments).

Within host parasite dynamics

In **chapter 5**, I observed positive correlations between the abundances of ectoparasite taxa and negative correlations between species of *Cichlidogyrus*. Positive relationships may be explained in two ways: *i)* they are true synergistic interactions, potentially resulting from parasite antigenic similarity that allows exploitation of immunomodulation by the other parasite *ii)* they result from being associated with same host ecological specialisation. Negative relationships may be due to competition, possibly related to parasite phylogenetic relatedness or on similarity in resource requirements. The direction and strength of parasite interactions did not differ between host species, suggesting that intrinsic host species traits do not influence parasite relationships, inconsistent with host specificity.

I also explored differences in reproductive activity of copepods (measured as the proportion of females carrying egg clutches) between host species, and observed no differences, among the wild caught species (**chapter 5**) and among the two lab-reared species of *Pundamilia* and their interspecific hybrids (**chapter 4**). This suggests no host specificity of copepod reproductive activity, although varying in infection prevalence and abundance.

CONCLUSION

In this thesis, I found support for parasites in contributing to host divergence, but not in initiating it. First, parasites are non-randomly distributed at least at three levels – gill microhabitat, host species, host lineages – indicating host specialisation and an opportunity for heterogenous parasite-mediated selection. Second, species differences in infection were temporally consistent, in line with prerequisites for parasite-mediated speciation. Third, when host species start to diverge in ecology, they also begin to accumulate differences in parasite communities, suggesting that differentiation in infection is a by-product of divergence rather than the opposite.