Within-host competition and diversification of macro-parasites

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Although competitive speciation is more and more regarded as a plausible mechanism for sympatric speciation of non-parasite species, virtually no empirical or theoretical study has considered this evolutionary process to explain intra-host diversification of parasites. We expanded the theory of competitive speciation to parasite species looking at the effect of macro-parasite life history on the conditions for sympatric speciation under the so-called pleiotropic scenario. We included within-host competition in the classical Anderson and May framework assuming that individuals exploit within-host resources according to a quantitative trait. We derived the invasion fitness function of mutants considering different distributions of individuals among hosts. Although the mutant fitness depends on parameters describing the key features of macro-parasite life history, and on the relative distributions of mutant and residents in hosts, the conditions for competitive speciation of macro-parasites are exactly the same as those previously established for free-living species. As an interesting by-product, within-host competitive speciation is expected not to depend on the aggregation level of the parasites. This theoretical pattern is confirmed by comparing the speciation rate of weakly and strongly aggregated monogenean parasites.

Keywords: sympatric speciation; competitive speciation; parasite duplication; aggregation; adaptive dynamics; monogenean parasites

1. INTRODUCTION

Parasites are regarded as good biological models for evolutionary studies, mainly because of their high species diversity [1], adaptive potential [2] and because most of their biotope—the host—is also a living and an evolving entity [3]. The different modes of parasite speciation are defined with respect to the level of interaction between parasite evolution and host diversification. While ‘host-switch’ implicitly requires previous host diversification, and ‘co-speciation’ literally emphasizes the contemporaneous diversification of a host and its parasite, ‘duplication’, that is speciation of parasites within a single host species, does not require host to diversify. Literature on diversification of parasites is overwhelmingly dominated by studies of host-switch and co-speciation events, which are believed to be the main modes of evolution of parasite species diversity [4,5].

Events of duplication have yet been shown to contribute to enhance parasite diversity of monogenean flatworms colonizing fish gills [6], Plasmodium species responsible for avian malaria [4] and Trypanosoma cruzi, the causal agent of the human American trypanosomiasis [7]. There are also more and more studies revealing the importance and variety of ecological interactions between parasites at the scale of the host body [8,9], including competition [10], facilitation by immuno-suppression [11] and inhibition by the elicitation of non-specific immune response [12]. With the concurrent theoretical and empirical realization that biotic interactions can lead to adaptive diversification in plants [13], fungi [14], algae [15], fishes [16] and various other taxa [17], one expects more adaptive duplication events to be reported as more attention would be given to this overlooked source of parasite diversity.

An important mechanism that could cause adaptive duplication is within-host competition for resources, as it can potentially lead to ‘competitive speciation’ [18]. Under such a scenario, competitive interactions at the host individual scale, i.e. within a parasite ‘infra-population’, lead to parasite diversification inside a population of conspecifics and sympatric host
individuals. Several lines of evidence point at the potential significance of such adaptive speciation in macro-parasites: within-host intraspecific competition has an impact on parasite life history [19], infra-populations are often genetically diverse [20], several congeneric species can live on the same host individual [21] and within-host competition influences the patterns of associations between such species [10]. Hypothetically, within-host competition could also contribute to micro-parasite diversity because infra-populations also are genetically diverse [7,22], and competition between strains is an important force driving parasite life-history evolution [23].

The theory of competitive speciation has been broadly extended in the last 10 years, using a wide range of models typically assuming that a quantitative trait is under disruptive selection generated by frequency and density-dependent intraspecific competition [24–25]. Some models consider that the ecological trait pleiotropically affects mate choice [26–28], while others explicitly describe the evolution of mating preferences according to the ecological trait under natural selection [26–30] or an additional neutral trait [26,31]. Despite criticisms [32], these studies have contributed to attract attention on the potential of intraspecific competition to induce adaptive diversification of non-parasite species. Surprisingly, no attempt has been made to account for the specific features of the parasite lifestyle, while parasites may represent 30 to 50 per cent of species diversity [1].

The main goal of this study was to investigate how conditions for competitive speciation depend on the typical features of macro-parasite life history by identifying when within-host competitive interactions can induce adaptive duplication. In this first attempt to expand the theory of competitive speciation to macro-parasites, we focused on the simplest case, the ‘pleiotropic scenario’ [33], whereby a ‘magic trait’ [24] under disruptive natural selection also contributes to non-random mating. Such ‘magic traits’ have been shown to be produced by a great variety of mechanisms in non-parasite species [34] and have been suggested to contribute to several events of within-host macro-parasite speciation [35]. We considered the exploitation of a gradient of resource, as the theory of competitive speciation is deeply rooted in the ecological literature on character displacement along such a gradient [28]. Examples of gradient of resource at the host body scale include fishes gill arches exploited by monogeneans [36], or nutrients exploited by gut helminths [37]. The scenario of speciation considered assumes that macro-parasites with different morphological or physiological phenotypes can exploit different parts of the gradient and that local competitive interactions contribute to shape the phenotypic distribution. According to the theoretical results developed for non-parasite species, one then expects the phenotypic distribution to become bimodal if local competition is strong enough [26]. We shall here identify whether or not considering key features of macro-parasite life history influences the conditions for such competitive processes to induce adaptive duplication, by comparing the theoretical results existing for non-parasite species [26,27] with the conditions identified from a general model that we shall design to include a description of the typical macro-parasite life history.

2. THE THEORY OF WITHIN-HOST COMPETITION AND ADAPTIVE DIVERSIFICATION OF MACRO-PARASITES

2.1. General approach

We expanded the theory of competitive speciation to macro-parasites using the model of Anderson & May [38,39]. To bridge the gap between this influential model and the theoretical literature on competitive speciation, we included within-host competition by assuming that individual competitive abilities depend on a quantitative trait, such as body mass or size [26–28]. We first established the population dynamic equations for the change in the number of individuals of two competing phenotypes. Considering these two phenotypes as mutant and resident, we then derived the fitness function of a mutant individual competing with residents already established in the host population. We finally performed an evolutionary analysis to reveal the condition for adaptive duplication of parasites, using the ‘adaptive dynamics’ approach previously applied to look at adaptive diversification of non-parasite species [26,27]. Importantly, we considered three hypotheses about the distributions of mutant and resident individuals among hosts, as such distributions were obviously anticipated to affect the fitness of the mutants. We assumed that mutant individuals tend to (i) colonize hosts at random, (ii) colonize the same hosts as the residents, or (iii) colonize hosts that are not already infected by the residents. These distributions are three simple alternatives to explore the role of spatial segregation in competitive interactions. They can potentially describe the distribution resulting from heterogeneities in host exposure/susceptibility to parasites or in pathological effects. For instance, heterogeneity in the spatial or temporal distribution of infective stages can determine the aggregated distribution of parasites in hosts ([40], p. 98), which would lead to a positive correlation between mutant and resident distributions. Alternatively, infection by resident individuals can lead to a pathological reduction of host mobility, and thus a lower susceptibility to infection by mutants, which would generate a negative correlation between mutant and resident distributions.

2.2. The extension of the Anderson and May's model to a polymorphic parasite population

We denoted \( H_{n_i n_j} (t) \) the number of hosts at time \( t \) that harbour \( n_i \) and \( n_j \) parasites with phenotype \( i \) and \( j \). We also let \( \mu (i) \), \( \mu (j) \) be the natural mortality rates of the two phenotypes, and \( \beta (i) \), \( \beta (j) \) their colonization rates. We described competition using two additional parameters: \( \alpha (i,j) = \alpha (j,i) \), the strength of within-host symmetrical competition between two individuals with phenotype \( i \) and \( j \), and \( K(i) \), \( K(j) \), the carrying capacities for individuals with phenotype \( i \) and \( j \).

For simplicity, we disregarded host demography and described transmission as a parasite pure immigration—
death process using standard modelling methods ([41], p. 140). Keeping \( n_j \) as a constant, variations in the number of hosts with \( n_i \) parasites are given by

\[
\frac{dH_{n_i}}{dt} = -\mu(i)(n_i)H_{n_i}(t) - (n_i + 1)H_{n_i+1}(t) + \beta(i)(H_{n_i-1}(t) - H_{n_i}(t)) - \alpha(i,i) \left( \frac{n_i^2}{K(i)} H_{n_i}(t) - \frac{(n_i + 1)^2}{K(i)} H_{n_i+1}(t) \right) - \alpha(i,j) \left( \frac{n_i n_j}{K(j)} H_{n_i+j}(t) - \frac{n_i (n_i + 1)}{K(j)} H_{n_i+1}(t) \right).
\]

(2.1a)

Competition is described here as in the existing theory of competitive speciation; its effects are assumed to reduce parasite within-host mortality and we did not consider alternative impacts that competition may have in reducing the rate of parasite establishment or reproduction.

A similar equation can be derived for the variations in the number of hosts with \( n_j \) parasites while keeping \( n_i \) as a constant

\[
\frac{dH_{n_j}}{dt} = -\mu(j)(n_j)H_{n_j}(t) - (n_j + 1)H_{n_j+1}(t) + \beta(j)(H_{n_j-1}(t) - H_{n_j}(t)) - \alpha(j,j) \left( \frac{n_j^2}{K(j)} H_{n_j}(t) - \frac{(n_j + 1)^2}{K(j)} H_{n_j+1}(t) \right) - \alpha(i,j) \left( \frac{n_i n_j}{K(j)} H_{n_i+j}(t) - \frac{n_i (n_j + 1)}{K(j)} H_{n_i+1}(t) \right).
\]

(2.1b)

Summing over \( n_i \) and \( n_j \) one can derive two equations for the change in the number of parasites with phenotype \( i \) and \( j \)

\[
\frac{dP(i)}{dt} = \sum_{n_i, n_j} \frac{dH_{n_i}}{dt} = -\mu(i)P(i) + \beta(i)H
\]

\[- \alpha(i,i) \sum_{n_i, K(i)} \frac{n_i^2}{K(i)} H_{n_i}(t) \]  

\[- \alpha(i,j) \sum_{n_i, K(i)} \frac{n_i n_j}{K(j)} H_{n_i+j}(t) \]  

(2.2a)

and

\[
\frac{dP(j)}{dt} = \sum_{n_i, n_j} \frac{dH_{n_j}}{dt} = -\mu(j)P(j) + \beta(j)H
\]

\[- \alpha(j,j) \sum_{n_i, K(j)} \frac{n_j^2}{K(j)} H_{n_j}(t) \]  

\[- \alpha(j,i) \sum_{n_i, K(i)} \frac{n_i n_j}{K(i)} H_{n_i+j}(t), \]  

(2.2b)

where \( P(i) \) and \( P(j) \) are the numbers of parasites with phenotype \( i \) and \( j \), and \( H \) is the number of hosts. Under the usual assumption of a fast equilibrium in the free-larval stage dynamics [41], \( \beta(i)H = \lambda \theta P(i)H/(\theta H + v) \) and \( \beta(j)H = \lambda \theta P(j)H/(\theta H + v) \), where \( \lambda \), \( v \) and \( \theta \) stand for the rates of production of larvae by adults, death of larvae and of host infection, respectively.

To further describe the competition between individuals \( \alpha(i,j) \) and \( K(i) \), \( K(j) \) have to be specified. We used standard assumptions of the theory of niche competition and competitive speciation [26–28]. A Gaussian distribution allows describing that competition is more intense between individuals with similar phenotypes

\[
\alpha(i,j) = e^{-((i-j)^2)/(2\sigma^2)},
\]

(2.3)

where \( \sigma^2 \) quantifies the intensity of competition: the larger \( \sigma^2 \), the stronger the competition for any given pair of phenotypes. A Gaussian distribution describes the carrying capacity of individuals with phenotype \( i \)

\[
K(i) = K(i^0)e^{-((i-i^0)^2)/(2\sigma^2)},
\]

(2.4)

where \( i^0 \) is the intermediate trait value associated with the maximal phenotypic carrying capacity \( K(i^0) \), and \( \sigma^2 \) specifies the width of the carrying capacity distribution according to individual phenotypes.

### 2.3. The invasive fitness function of competing mutant macro-parasites

To analyse the evolution of the quantitative trait determining the use of resources by competing macro-parasites, we searched for the fitness function of a mutant with phenotype \( m \) invading a population of residents with phenotype \( r \). Such fitness function can be derived from equations describing the ecological interactions between mutant and resident individuals [26–28]. We used equations (2.2a) and (2.2b) to describe the dynamics of such interactions, assuming that mutant individuals are rare, and that residents are at their population dynamics equilibrium. The first assumption allows neglecting competition between mutants and simplifying the description of competition between mutant and resident individuals. Indeed, the number \( n_m \) of mutants in any given host can only be 0 or 1, and further considering that, at all times, \( H_m(t) = H_m^*, \) the last sum \( S(m,r) \) in equation (2.2a) becomes

\[
S(m,r) = \sum_{n_m, n_r} n_m n_r H_{n_m, n_r} \approx P(m) \sum_{n_r} n_r H_{n_r}^* p(n_r),
\]

(2.5)

where \( p(n_r) \) is the probability for a mutant parasite to enter a host already infected with \( n_r \) resident parasites. This approximation involves that the number of hosts harbouring \( n_m \) and \( n_r \) mutant and resident parasites is proportional to the number of mutants, because this number is the limiting factor in the co-occurrence of both types within a host.

Using equation (2.2a) and the earlier-mentioned assumptions, one can derive a general expression of
Table 1. Fitness functions under different assumptions of distribution of mutant and resident individuals. \( p_d(n) \) is the probability for a mutant individual to parasitize a host already harbouring \( n \) resident individuals. \( S_d(m,r) \) is the sum describing interaction between resident and mutant individuals as defined by equation (2.5). \( F_d(r) \) is defined as in equation (2.10). \( p_d(n), S_d(m,r) \) and \( F_d(r) \) are given for three distributions of mutant individuals: a random distribution \( (d = 1) \), a co-aggregated distribution \( (d = 2) \) and an inversely aggregated distribution \( (d = 3) \). The standard theoretical result for non-parasite species [26,27] appears in the last row of the table.

<table>
<thead>
<tr>
<th>mutant distribution</th>
<th>( p_d(n) )</th>
<th>( S_d(m,r) )</th>
<th>( F_d(r) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>random ( (d = 1) )</td>
<td>( \frac{1}{H} )</td>
<td>( P(m) \left( \frac{P(r)^*}{H} \right) )</td>
<td>( \frac{k}{k+1} (cK(r) - 1) )</td>
</tr>
<tr>
<td>co-aggregation ( (d = 2) )</td>
<td>( \frac{n}{P(r)^*} )</td>
<td>( P(m) \left( \frac{P(r)^*}{H} \frac{k+1}{k} + 1 \right) )</td>
<td>( cK(r) )</td>
</tr>
<tr>
<td>inverse-aggregation ( (d = 3) )</td>
<td>( 1 - (\frac{n}{P(r)^*}) )</td>
<td>( P(m) \left( \frac{P(r)^*}{H} \frac{k+1}{k} - 1 \right) )</td>
<td>( \frac{H(k(k+1))(cK(r) - 1) - cK(r)}{H - 1} )</td>
</tr>
<tr>
<td>non-parasite ( (d = 4) )</td>
<td>—</td>
<td>—</td>
<td>( cK(r) )</td>
</tr>
</tbody>
</table>

the invasion fitness of a mutant \( m \) in a population of residents \( r \)

\[
f(m, r) = \frac{dP(m)}{P(m)dt} = -\mu(m) + \frac{\lambda \theta H}{\theta H + v} \]

\[
- \alpha(m, r) S(m, r) \frac{K(m)}{P(m)}. \tag{2.6}
\]

Specifying the probabilities \( p_d(n) \) appearing in \( S(m, r) \) would thus allow us to complete the definition of the fitness function.

We used the traditional assumption that the aggregated distribution of residents is described by a Negative Binomial Distribution [38]. We then worked out three expressions of \( p_d(n) \), noted \( p_d(n) \), whereby mutant and residents distributions are either independent \( (d = 1) \), positively \( (d = 2) \) or negatively \( (d = 3) \) correlated. When \( d = 1 \), the probability for a mutant to enter a host harbouring \( n \) resident parasites is proportional to the frequency of hosts carrying \( n \) residents. When \( d = 2 \), this probability is proportional to the number \( n \) of residents. When \( d = 3 \), the probability is inversely proportional to the number \( n \) of residents. Normalizing these probabilities, we found the expressions \( p_d(n) \) appearing in table 1. Since residents are assumed to be distributed in hosts according to a negative binomial distribution

\[
\sum n^2 H_n(t) = \frac{P(r)^2}{H} \left( \frac{k+1}{k} \right) + P(r), \tag{2.7}
\]

where \( k \) is the usual aggregation parameter [38].

One can then express \( S(m, r) \) with respect to \( P(r)^* \), the number of resident parasites at their population dynamic equilibrium in absence of mutant (table 1). This equilibrium level, \( P(r)^* \), can be derived from equation (2.2a), which now reads

\[
\frac{dP(r)}{dt} = -\mu(r)P(r) + \frac{\lambda \theta H}{\theta H + v} P(r) \]

\[
- \frac{\alpha(r, r)}{K(r)} \left( \frac{P(r)^2}{H} \frac{k+1}{k} + P(r) \right). \tag{2.8}
\]

and is given by

\[
P(r)^* = H \left( \frac{k}{k+1} \right) (c(r)K(r) - 1), \tag{2.9}
\]

with \( c(r) = -\mu(r) + (\lambda \theta H)/(\theta H + v) \).

Substituting \( P(r)^* \) into \( S(m, r) \), the fitness functions defined for the three mutant distributions, can be rewritten (from equation (2.6)) in the general form

\[
f_d(m, r) = c(r) - \frac{\alpha(m, r)}{K(m)} F_d(r), \tag{2.10}
\]

where \( F_d(r) \) are as defined in table 1.

Interestingly, the mutant fitness function derived for non-parasite species [26,27] can also be written in that form

\[
f_d(m, r) = c - \frac{\alpha(m, r)}{K(m)} [cK(r)], \tag{2.11}
\]

where \( c \) is usually thought not to depend on the individual phenotype. We thus assumed that \( c \) is a constant for the three fitness functions derived for parasite species, to ease the comparison with non-parasite species.

A first conclusion (from equation (2.10) and definitions of \( F_d(r) \) in table 1) is that, when the mutant and resident distributions are positively correlated \( (d = 2) \), the fitness functions for parasite and non-parasite species are equivalent. The only difference between them lies in the definition of \( c \), which is related to parameters reflecting parasite or non-parasite life histories. In contrast, when mutant distribution is random \( (d = 1) \) or negatively correlated \( (d = 3) \) with the distribution of residents, the mutant parasite fitness functions depend on the aggregation level of the residents. It can also be shown that \( F_3(r) = F_2(r) > F_1(r) > F_0(r) \) so that \( f_3(r) = f_2(r) < f_1(r) = f_0(r) \), which means that, as expected, the more segregated the mutant and resident are, the fittest the mutant since it suffers less competition from resident.

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2.4. Evolutionary dynamics and adaptive duplication of macro-parasites

The ‘adaptive dynamics’ approach [42] allows identifying evolutionary singularities, that is trait values where the selection gradient vanishes. Four evolutionary properties of these singularities are usually assessed: evolutionary stability, convergence stability, invasion potential and mutual invadibility. These properties are assessed using rules on the derivatives of the fitness function with respect to \( m \) and \( r \). We thus worked out these derivatives and properties using the general form of the fitness function equation (2.10), assuming that \( c \) does not depend on the individual phenotype (as usually done for non-parasite species [26]), and describing competition according to equations (2.3) and (2.4). Later, we report the conditions for the evolutionary singularity to have the different properties evoked earlier. Details of the mathematical derivation of these results and numerical illustrations of the analytical results are given in the electronic supplementary material.

Evolutionary singularities can be identified by solving

\[
\delta f_d(m, r) \left|_{m=r=s} \right. = -\frac{F_d(s^*)}{K(s^*)} \left( \frac{s^* - s_0}{a} \right) \sigma_K^2 = 0. \tag{2.12}
\]

Since \( F_d(s^*) \neq 0 \) (\( \forall d \in [1; 4] \)) (see the electronic supplementary material), there is only one evolutionary singularity: \( s^* = s_0 \), which is exactly the same for all macro-parasite fitness functions \( (d = 1,2,3) \), and for the non-parasite species \( (d = 4 \) and \([26,27]) \). It simply corresponds to the value of the trait providing the maximal amount of resource \( (K(s_0)) \), and it is worth mentioning that this evolutionary singularity does not depend on the distribution of the mutants nor on the level of aggregation of the residents.

Such an evolutionary singularity is evolutionary stable if

\[
\delta^2 f_d(m, r) \left|_{m=r=s} \right. = \frac{F_d(s^*)}{K(s^*)} \left( \frac{1}{\sigma_a^2} - \frac{1}{\sigma_K^2} \right) < 0. \tag{2.13}
\]

Since \( F_d(s^*) > 0 \) (\( \forall d \in [1; 4] \)) (see the electronic supplementary material), whatever the fitness function, the condition for the evolutionary singularity \( s^* = s_0 \) to be an evolutionary stable strategy is \( \sigma_a^2 > \sigma_K^2 \). This condition is biologically meaningful as large \( \sigma_a^2 \) means that residents \( s^* = s_0 \) compete with (mutant) individuals having different phenotypic values, which can prevent invasion. Instead, if \( \sigma_a^2 \) is small, residents do not exert any competition pressure on mutants, which can then invade. Again, the results we obtained for macro-parasites \( (d = 1,2,3) \) are similar to the one obtained for non-parasite species \( (d = 4 \) and \([26,27]) \).

The evolutionary singularity is a convergent stable strategy (CSS) if

\[
\frac{\partial^2 f_d(m, r)}{\partial^2 r} \left|_{m=r=s} \right. = \frac{\partial^2 f_d(m, r)}{\partial^2 m} \left|_{m=r=s} \right. = \frac{1}{K(s^*)} \left( \frac{2F_d(s^*)}{\sigma_a^2} - \frac{\partial^2 F_d(r)}{\partial^2 r} \right) \left|_{m=r=s} \right. > 0. \tag{2.14}
\]

Evaluating this inequality according to the different function \( F_d \) and their second derivative with respect to \( r \), we obtained the conditions for the evolutionary singularity to be a CSS for each of the four fitness functions (see the electronic supplementary material).

When the mutant distribution is positively correlated with the resident distribution \( (d = 2) \), the expression of this condition is exactly the same as for non-parasite species \( (d = 4) \) and, remarkably, it does not depend on the aggregation parameter. In contrast, for the other two types of mutant distribution, these expressions depend on the details of the parasite life history and, especially, on the aggregation level. Despite these differences, \( s^* \) remains always an evolutionary convergent strategy (see the electronic supplementary material), which means that \( s^* \) can be gradually reached through a sequence of adaptive mutations with small phenotypic effects.

The evolutionary singularity is an invasive strategy if

\[
\frac{\partial^2 f_d(m, r)}{\partial^2 r} \left|_{m=r=s} \right. = \frac{1}{K(s^*)} \left( \frac{F_d(s^*)}{\sigma_a^2} - \frac{\partial^2 F_d(r)}{\partial^2 r} \right) \left|_{m=r=s} \right. > 0. \tag{2.15}
\]

Evaluating this inequality for the different functions \( F_d(r) \), one obtains the conditions for the evolutionary singularity \( s^* \) to be able to invade in a non-gradual way (see the electronic supplementary material).

Again, those conditions are exactly the same when the mutant distribution is positively correlated with the resident distribution \( (d = 2) \) and for non-parasite species \( (d = 4) \). When mutant and residents distributions are different \( (d = 1 \) and \( 3) \), the conditions depend on the parameter related to macro-parasite life history (see the electronic supplementary material). These conditions are analogous to the conditions for \( s^* \) to be convergent stable, except that, in the first term, \( \sigma_a^2 \) is replaced by \( \sigma_a^2 \). Again, those conditions are always verified (see the electronic supplementary material).

Evolution can thus lead to the establishment of \( s^* \) in a non-gradual way, typically by the fixation of a mutation of a large effect.

Finally, a protected polymorphism (PP) could appear in the vicinity of the singular strategy if

\[
\frac{\partial^2 f_d(m, r)}{\partial^2 r} \left|_{m=r=s} \right. + \frac{\partial^2 f_d(m, r)}{\partial^2 m} \left|_{m=r=s} \right. = \frac{1}{K(s^*)} \left( \frac{2F_d(s^*)}{\sigma_a^2} - \frac{\partial^2 F_d(r)}{\partial^2 r} \right) \left|_{m=r=s} \right. > 0. \tag{2.16}
\]

Not surprisingly, these conditions are exactly the same when the mutant distribution is positively correlated with the resident distribution \( (d = 2) \) and for non-parasite species \( (d = 4) \), and they are always satisfied (see the electronic supplementary material). When mutant and resident distributions are different \( (d = 1 \) and \( 3) \), the condition also always holds (see the electronic supplementary material). A pair of mutations

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with larger and smaller trait values than $s^*$ can then invade each other, so that a PP can potentially be established, leading to two sets of individuals exploiting two opposite parts of the resource gradient.

2.5. Branching point

Branching points are paradigmatic features of the adaptive dynamics background, especially when looking at adaptive diversification and speciation. They are points around which a monomorphic population can become polymorphic because frequency- and density-dependent ecological interactions generate a disruptive selection pressure. In addition, when the ecological trait under disruptive selection pleiotropically affects pre-zygotic isolation, adaptive ecological diversification almost systematically leads to adaptive speciation [26,27]. Conditions for adaptive diversification under such a scenario are thus identified by identifying the range of parameter values where $s^*$ is a ‘branching point’. For $s^*$ to be a ‘branching point’, it has to be CSS with the potential to invade (IP) in a non-gradual way, and to be a non-evolutionary stable strategy (non-ESS) around which a PP can appear. The first two properties (CSS and IP) ensure that evolution can lead to $s^*$. The last two properties (non-ESS and PP) allow for diversification around this strategy after it has been reached.

Whatever the assumption about the mutant distribution among hosts, the conditions for the singular strategy to be convergent stable, to be able to invade and for a PP to appear always hold. Thus, the only requirement for $s^*$ to be a branching point is to be an evolutionary stable strategy, which, as previously stated for non-parasite species, requires $\sigma_n^2 < \sigma_X^2$ [26,27].

The conditions for adaptive duplication owing to within-host competitive interactions are thus the same as the conditions for competitive speciation of non-parasite species. An important by-product of this conclusion is that, despite the variations of the fitness function originating and six species originated from other types of speciation (figure 1). Intra-host speciation or duplication events were depicted using the host–parasite tangle tree representation, and the robustness of the intra-host speciation (duplication events) was tested by bootstrap percentage on randomized parasite and host phylogenies (see Šimková et al. [6] for details). We then used the MacnOCAIC program [47], derived from CAIC [48] to test our prediction of independence between the aggregation level and speciation of parasites. MacnOCAIC is designed specifically for studies of diversification and uses a basic approach based on phylogenetically independent contrasts. MacnOCAIC allows using species richness as a variable in a comparative analysis to estimate whether a trait (here aggregation) is associated with high speciation rates [49]. We used a widely acknowledged measure of the aggregation level of parasites [50]; the ratio of the variance of abundance [$V(M)$] of a parasite's distribution to its mean abundance ($M$). We used this measure instead of the parameter $k$ of the negative binomial, as the latter can be biased when estimated on different species with obvious different sample sizes [51].

We regressed the natural log of the CladeBigX/CladeSmallX ratio where CladeBigX is the species richness of the sister clade with larger aggregation, for each node of the phylogeny, and CladeSmallX is the species richness of its sister clade with lower aggregation. The ln(CladeBigX/CladeSmallX) was regressed against standardized contrasts for $\text{Var}(M)/M$, and we found no significant correlation between monogenean aggregation and diversification ($p = 0.40, n = 16$; figure 2). In other words, clades with higher species richness are not characterized by smaller or larger aggregation than their sister clades. Despite the potential bias
when using parameter $k$, we performed the same comparative analysis on $k$ estimates and found a similar pattern, suggesting that this conclusion is robust.

4. DISCUSSION

We investigated the conditions for within-host adaptive diversification by expanding the theory of competitive speciation to macro-parasite species, and by confronting our theory to a comparative analysis of speciation rates in monogenean parasites.

Although the invasion fitness depends on parameters describing key features of the macro-parasite life history, we showed that it is typologically equivalent to those previously derived for non-parasite species as long as the mutants tend to colonize the same hosts as the residents. In contrast, when mutants randomly colonize host individuals, or when they tend to colonize less infected hosts, the fitness functions of parasites and non-parasites are no longer similar. As expected, differences between the distribution of mutants and residents promote mutant invasion. This is consistent with the
The well-known effect of spatial heterogeneity to promote coexistence between competitive species of mammals [52], birds [53], plants [54], fungi [55] as well as macro-parasites [56].

Regardless of differences in fitness functions obtained for various distributions of the mutant among hosts, the conditions for within-host adaptive diversification remain the same and are exactly the same as for competitive diversification of non-parasite species [25]. This suggests that previous results on the influence of interspecific competition on non-parasite species diversity are robust to the life history of the organisms being considered. This leads to the unanticipated conclusion that within-host adaptive diversification of macro-parasites does not depend on the level of aggregation among hosts. Such a conclusion contrasts with the recognized effects of aggregation on population dynamics [38,44] interspecific competition [43,57], and the evolution of both host and parasite [40]. Interestingly, the comparison of speciation rates between clades of monogeneans with low and high levels of aggregation was consistent with this theoretical prediction. Although the failure of detecting an effect of aggregation may entail a lack of statistical power, this finding is consistent with those of Šimková et al. [58] and suggests that intra- and interspecific aggregation affect the strength of population regulation of monogeneans, but with no incidence on their diversification through competitive speciation.

We used a simple strategic ‘core’ model [59] that allows accounting for general features of macro-parasite lifestyle to specify the conditions for within-host competition to cause adaptive diversification. Although the description of the parasite life history was consistent with the lifestyle of the monogeneans used to confront our theory to existing phylogenetic studies, there are several lines along which to improve our theory and our understanding of the role of within-host competition in parasite diversity.

Our evolutionary analysis relied on a phenotypic approach, which does not account for sexual reproduction. The conditions for the population to split into subpopulations of different phenotypes might thus not strictly correspond to the conditions for within-host duplication [28]. However, when considering a ‘magic’ trait, whereby assortative mating between phenotypically similar individuals appears as a by-product of ecological specialization, the conditions for competitive speciation established by individual-based models (including sexual reproduction and the genetic underlying the ecological trait) do match the conditions for the ecological phenotype diversification [26,27]. Because the size of the haptors used by monogeneans to attach on gills is thought to be such a magic trait [35], our results indeed inform on duplication events. For parasites developing within the host body, such as intestinal nematodes, chemical communication can influence encounters between conspecific individuals as well as encounters between individuals of different species [60,61]. Such chemical communication has also been observed for non-parasite [62] and plant parasite [63] species closely related to nematodes. Potentially, such communication could thus evolve to limit hybridization between incipient species of intestinal nematodes, as observed in the plant–parasite nematode Radopholus similis [64]. According to the general theory of reinforcement [24], the conditions for parasite duplication involving the evolution of assortative mating based on such chemical communication are likely to be more limited, even if the post-zygotic isolation owing to intraspecific competitive interactions provides the required selective pressure [26]. It would thus be worth expanding the theory to account for the evolution of such a ‘mating trait’ as previously done for non-parasite organisms [26]. Identifying the condition for such mating clue to evolve would undoubtedly provide new insights into the idea that the present chemical signalization between conspecific individuals represents the ghost of past competition.

The theory we developed does not consider any effect of macro-parasites on their hosts. Such an assumption is consistent with the common view that macro-parasites are less virulent than many micro-parasites, such as bacteria [65], viruses [66] or protozoans [67]. Nevertheless, macro-parasites can affect their host survival and reproductive rates either on their own [68] or through co-infection with micro-parasites [69]. To expand the theory to account for parasite virulence is likely to produce further stimulating results, especially about the role of aggregation. Indeed, as the most heavily infected hosts are where the intraspecific competition is the highest, they also are where disruptive selection is the strongest. If such hosts were to die because of their heavy rate of infection, aggregation would have an adverse effect on adaptive diversification. Potentially, the virulence of the resident population could thus freeze further evolution. Such a possibility of ‘frozen evolution’ has been widely overlooked, while it could be relevant to the evolution of any other life-history trait of macro-parasites. We thus anticipate that accounting for virulence would limit adaptive diversification and duplication induced by within-host competition, a conclusion that is likely to depend on the level of co-distribution between mutants and residents.

The model we used provides a proper theoretical background to better understand the origin and evolution of ‘species flocks’ observed in single host species.
such as fish gill monogeneans [70,71] or gastrointestinal helminths [21,70] that interact along a gradient of resource. However, some macro-parasites show much stronger spatial segregation as they colonize different locations within the host body. For instance, adult flatworms such as *Schistosoma* settle in various parts of the blood vessel network [72], while adults of the filarial roundworms *Onchocerca* are found in tissues such as the skin, muscles, joints or blood vessels [73]. Here again, expanding the theory of competitive speciation to macro-parasite could stimulate research on parasite evolution. In a recent attempt, Thibert-Plante & Hendry [74] have explored adaptive diversification when individuals compete for a bimodal resource distribution. They looked at how competition and mate choice interact with the discreteness of the environment to allow for sympatric speciation. Although the initial phenotypic distribution used mimicked the colonization of a new host with different potential locations and no initial adaptation to any of them (rather than the adaptation to a new location within the host body), this framework could allow questioning how within-host interactions can promote or impede adaptive diversification. This would could account for the specificity of macro-parasites, and could lead to an original scenario of competitive speciation as parasites using one of the peaks (or locations) are undoubtedly eliciting an immune response. Such a response to macro-parasites typically involves the TH2 immune response [75] and is likely to be non-specific [76]. It would then impose a negative density-dependent effect on mutants trying to colonize a new location, and therefore increase selection against mutants that are already likely to suffer from a rare phenotype mating disadvantage [28,77]. The indirect interactions owing to the non-specific immune response may then impose an additional form of stabilizing selection opposing within-host adaptive diversification.

In conclusion, this study is the first attempt to expand the theory of competitive speciation to macro-parasites and to better understand duplication as an adaptive response to within-host competition for resources. We have enlightened how expanding the existing theory to macro-parasites can enhance the general theory of adaptive speciation and provide novel conceptual insights into our nascent understanding of the origin of macro-parasite diversity [71]. Hopefully, this will help parasitologists to overcome difficulties in looking at the variability of life-history traits and interactions at the intraspecific level, where the selective pressures explaining parasite diversification can be identified. To improve predictions about the adaptive evolution of macro-parasites requires developing more specific models corroborated by a fine understanding of their life history and interactions with the host. Importantly, most theoretical contributions about the ecology, evolution and control of macro-parasites infecting wild animals, livestock or human were obtained using Anderson & May’s model [39]. The theory we developed, and the improvements we discussed, thus provide the natural framework to understand the influence of within-host competitive interaction on life-history evolution and speciation of macro-parasites, as well as to design evolutionary-proof controls to reduce their impact on livestock and human populations [78].

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