

# Genetic and environmental determinants of host use in the trematode *Maritrema novaezealandensis* (Microphallidae)

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## SUMMARY

Factors constraining host specificity are poorly understood. Intraspecific variation in host preferences in generalist parasites may reveal which factors affect patterns of host use, and thus the evolution of specialization. Here, laboratory experiments examined genetic variation in host preferences and the effect of a refugium against infection on host use. Firstly, 6 cercarial clones of the trematode *Maritrema novaezealandensis* (ranging widely in heterozygosities) were exposed simultaneously to 2 alternative hosts, the amphipods *Heterophoxus stephensi* and *Paracalliope novizealandiae*, to assess host preferences and fitness correlations with parasite heterozygosity. All clones showed a distinct preference for *H. stephensi*, though the extent of this preference varied among clones. No clear association was found between heterozygosity and either parasite infection success or preference for a particular host. Secondly, cercariae were exposed to the same 2 amphipods in both the presence and absence of sand (refugium for *H. stephensi*). Without sand, infection levels were significantly higher in *H. stephensi* than in *P. novizealandiae*. With sand, *H. stephensi* was able to hide, offsetting the parasite's intrinsic preferences for this host. These results demonstrate the existence of genetic variation in host preferences, as well as the effect of environmental variables on observed patterns of host use.

**Key words:** host specificity, genetic variation, heterozygosity fitness correlation, amphipod, trematode, *Maritrema novaezealandensis*, *Heterophoxus stephensi*, *Paracalliope novizealandiae*.

## INTRODUCTION

The forces shaping the evolution of host specificity in parasites remain poorly understood, despite the fact that they underpin key aspects of parasite epidemiology, such as the probability of local extinction or the likelihood of emerging diseases following biological invasion (Daszak *et al.* 2000; Cleaveland *et al.* 2001; Poulin, 2007). Simple ecological, physiological and behavioural 'filters' can sometimes explain patterns of host use (Euzet and Combes, 1980). Indeed, among potential host species, there is variation in both the rates at which parasites encounter the hosts and their degree of compatibility with the parasite (Combes, 2001; Poulin, 2007). Recently, experimental infections have proven very useful in distinguishing between the roles of encounter and compatibility when determining which host species are actually used by a parasite (Poulin and Keeney, 2008; Detwiler and Minchella, 2009).

However, with rare exceptions (e.g. Downes, 1986; Jaenike and Dombeck, 1998; Paterson, 2005), host exposure experiments have not been used to explore variation in host preferences within particular parasite species. Here, 'preference' is used to mean greater infection success in one host species than in others

available at the same time; we do not imply that infective stages choose between hosts, but that variation in their behaviour can lead to differential utilization of available hosts. In generalist parasites, potentially capable of using several of the locally available host species, intraspecific variation in host preferences can shed some light on the factors affecting patterns of host use, and thus on the eventual evolution of specialization. On one hand, there may be different host preferences among different parasite genotypes. If certain parasite genotypes perform better in one host species than in another, selection should favour preferences for that host (Fry, 1990; Ward, 1992; Poulin, 2007). The preferred host may differ among parasite genotypes, leading to variation within a parasite population in patterns of host use. The bet-hedging infection strategy (Fenton and Hudson, 2002) suggests that parasites can increase their infection success through variation amongst closely related individuals. On the other hand, environmental factors can also influence these patterns. For instance, the presence or absence of particular microhabitats could determine whether or not refuges against infection are available to certain hosts and override any genetically determined host preferences.

We examined both genetic variation in host preference and the impact of host refuges on host susceptibility in the trematode *Maritrema novaezealandensis* (Microphallidae), a common parasite in New Zealand intertidal communities (Koehler and

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Poulin, 2010). The adult stage has been described from the red-billed gull, *Larus novaehollandiae scopulinus* (Martorelli *et al.* 2004), but probably uses a variety of other seabirds, shorebirds and waterfowl as definitive hosts. The first intermediate host, the mud snail *Zeacumantus subcarinatus*, a gastropod common to soft sediment bays, produces cercariae, genetically identical replicates that are the free-living infective stage of the parasite. After leaving the snail, cercariae penetrate the cuticle of the intermediate crustacean hosts. Hundreds of cercariae can be produced at a time, actively swimming around for up to 12 h until they come into contact with a second intermediate host or die (A. Koehler, personal observation). No studies have been conducted on possible host-seeking mechanisms for *M. novaezealandensis* such as chemical cues. There is, however, evidence of photophobic behavioural response to light (A. Koehler, unpublished data).

Importantly, *M. novaezealandensis* cercariae show very little host specificity and can use a broad range of crustaceans as second intermediate hosts, including crabs, amphipods and isopods (Koehler and Poulin, 2010). This trematode is an ideal model for investigations of intraspecific variation in host preferences not only because it can use several crustacean species, but also because specific microsatellite markers are now available for this species (Keeney *et al.* 2006; Molecular Ecology Resources Primer Development Consortium *et al.* 2009). These can be used to distinguish between clones of the parasite, and to estimate the heterozygosity of each genotype. Although still controversial, correlations between heterozygosity at neutral loci and various fitness measures (heterozygosity-fitness correlations, or HFCs) have been reported for a wide range of organisms (Hansson and Westerberg, 2002; Chapman *et al.* 2009). If heterozygous trematodes generally outperform homozygous ones, then we might expect a wider range of host preference in heterozygous genotypes and a more specific preference in mostly homozygous ones, a prediction based on Van Valen's (1965) niche variation hypothesis that can be tested with *M. novaezealandensis* clones.

Two amphipod host species of *M. novaezealandensis* were considered in this study. The first, *Paracalliope novizealandiae* (Eusiridae), is a small (body length approximately 2.5 mm (Barnard, 1972)) and very abundant amphipod living among seaweed, where it feeds by grazing on epiphytic microalgae. The second, *Heterophoxus stephensi* (Phoxocephalidae), is a larger amphipod (body length approximately 6 mm (Hurley, 1954)) whose microhabitat overlaps with the first species, though it lives burrowed in the uppermost layer of sediment. Phoxocephalids are considered detritivores and opportunistic predators that occasionally spend time in the water column (Oakden, 1984). Independently of

any genetic preferences of the parasite for either amphipod species, one key environmental factor could change patterns of host use: the presence of suitable sediments (sand-silt mixture) could determine whether or not *H. stephensi* benefits from a potential refuge against infective cercariae. In this system, this single environmental factor could cause a switch in the parasite's intrinsic host preferences.

The present study used complementary experiments to investigate genetic variation in host preferences and the effect of a refugium against infection on patterns of host use by the trematode *M. novaezealandensis*. The first experiment compared the relative preferences of different clones of *M. novaezealandensis* for each of the two amphipod hosts during simultaneous exposures. Cercariae, released from snails bearing unique clonal parasite genotypes, give an accurate measurement of host preferences, which may vary among genotypes. This also allowed us to determine if the strength of any host preference varied with heterozygosity. The second experiment compared patterns of host use by *M. novaezealandensis* in the presence and absence of sediment, to determine whether environmental conditions can override any intrinsic host preference by the cercariae. Specifically, we tested the prediction that the relative use of one host species (*H. stephensi*) by the parasite will decrease markedly when a refuge against infection is provided for that host.

## MATERIALS AND METHODS

### Field collection

In order to establish that *M. novaezealandensis* is a natural parasite of both amphipods, sympatric populations of *H. stephensi* and *P. novizealandiae* were sampled from Papanui Inlet, Otago Peninsula, Dunedin, New Zealand (45°51'09"S, 170°41'51"E) on 22 February 2010. In total, 88 *H. stephensi* and 100 *P. novizealandiae* were haphazardly collected using an aquarium net in approximately 50 cm of water. Amphipods were sexed, dissected and the number and developmental stage of *M. novaezealandensis* were recorded. Measurements of amphipod size were not included in this study as previous studies of *M. novaezealandensis* and *P. novizealandiae* concluded that size of amphipods did not have a significant effect on intensity of infections (Fredensborg and Poulin, 2005; Bryan-Walker *et al.* 2007).

### Genotype experiment

Simultaneous exposures were used to compare the relative preferences of different clones of *M. novaezealandensis* for each amphipod host. Amphipods were collected from Hooper's Inlet, Otago Peninsula,

Dunedin, New Zealand (45°51'13"S, 170°40'12"E) 2 days prior to infection, in July 2009. The snail host for *M. novaezealandensis* is absent from this inlet, therefore amphipods from this site were uninfected, as previous studies have confirmed (Fredensborg *et al.* 2004; Bryan-Walker *et al.* 2007). After identifying snails harbouring only single infections (by genotyping subsets of cercariae from each snail using microsatellite markers), each clonal parasite infection was genotyped using 32 polymorphic microsatellite markers, and heterozygosity (proportion of heterozygous loci) was determined (see Keeney *et al.* 2006; Molecular Ecology Resources Primer Development Consortium *et al.* 2009). No loci showed significant deviations from Hardy-Weinberg equilibrium, and no significant linkage disequilibrium was found in any pair of these loci (Molecular Ecology Resources Primer Development Consortium *et al.* 2009). Three clones with lower heterozygosities (1 with 37.50% and 2 with 43.75%) and 3 with a higher heterozygosity (all with 56.25%) were chosen.

Experiments were carried out in 55 mm diameter plastic Petri dishes filled with 10 ml of seawater, with 10 replicates for each of the 6 clones. Five *H. stephensi* and 10 *P. novizealandiae* were added to each dish. Twice as many *P. novizealandiae* were added because *P. novizealandiae* are more abundant in their natural environment. Approximately 100 cercariae (dosage based on prior infection trials) were added to the respective dishes. In an attempt to standardize conditions between experiments, dishes were placed in a dark enclosure at room temperature for approximately 18 h. Amphipods that had died during this period were excluded from both this experiment and the next, as their cercariae could not be accurately quantified. All remaining amphipods were euthanized by severing the ventral nerve cord, immediately dissected and the number of cercariae successfully infecting each host was recorded. The various tissues in which cercariae were found were not recorded. Cercariae left in each dish were not counted after the experiments, because by that time they would have been dead and decomposing. Reviews of larval parasite predation literature (Thieltges *et al.* 2008; Johnson *et al.* 2010) have found no specific incidents of amphipod hosts consuming cercariae. It is unlikely that *P. novizealandiae* would prey on cercariae due to its herbivorous diet and if *H. stephensi* did consume cercariae we assume they would have done so equally across replicates.

#### Environment experiment

This experiment was carried out in twenty 55 mm diameter plastic Petri dishes. Half were filled with 10.5 g of sand from Hooper's Inlet (enough to cover

the bottom of the dish), and the other 10 dishes were without sand. Both sets of dishes were filled with 10 ml of seawater. As in the previous setup, 5 *H. stephensi* and 10 *P. novizealandiae* were added to each dish. To remove any genetic effect, cercariae were pooled from 12 *M. novaezealandensis*-infected snails and approximately 100 cercariae were added to each dish. Amphipods were then processed as in the prior experiment, followed by dissections and data collection.

#### Statistical analyses

Data could not be transformed to meet assumptions of normality, therefore the non-parametric Kruskal-Wallis test (K-W), which is based on ranks, was used to assess the statistical significance of differences between groups. Hsu's *post hoc* test was used to determine which groups were significantly different. For the genotype experiment, the ratio of parasite mean abundance between the two host species (*H. stephensi* to *P. novizealandiae*) was computed for each replicate and used to assess differences in host preferences between clones. Mean abundance was calculated as the total number of parasites divided by the total number of hosts, for each amphipod species and for each replicate (Petri dish). By using the ratio of parasite mean abundance in our analysis, we account for any disparities that arise from using 10 *P. novizealandiae* and 5 *H. stephensi*. Parasite prevalence (percentage of infected hosts) and parasite mean intensity (total number of parasites divided by the total number of infected hosts) were also calculated.

## RESULTS

### General results

There was no significant difference in parasite abundance between sexes for either *H. stephensi* or *P. novizealandiae* in either the field survey, the genotype experiment or the environmental refuge experiment (all  $P > 0.05$ ) therefore data from both sexes were pooled. From the field collection, natural infection prevalence of *M. novaezealandensis* was 17% (mean intensity =  $1.60 \pm 0.32$ ) in *H. stephensi* and 71% (mean intensity =  $4.16 \pm 0.39$ ) in *P. novizealandiae*. Mature metacercariae were found in both amphipod species. For the genotype experiment the mortality rate was 9.7% for *H. stephensi* and 4.0% for *P. novizealandiae*. For the environmental refuge experiment the mortality rate for *H. stephensi* was 3.0% and that for *P. novizealandiae* was 2.5%.

### Genotype experiment

When the 6 clones were grouped into either high or low heterozygosity categories, a significantly higher

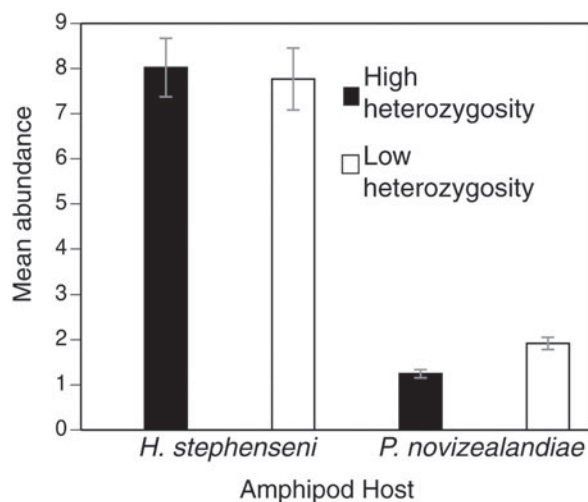


Fig. 1. Mean ( $\pm$ S.E.) abundance of *Maritrema novaezealandensis* in *Heterophoxus stephenseni* and *Paracalliope novizealandiae* when the 6 clones were grouped into either high or low heterozygosity categories. A significant difference was found between the high and low heterozygosity groups of *P. novizealandiae*.

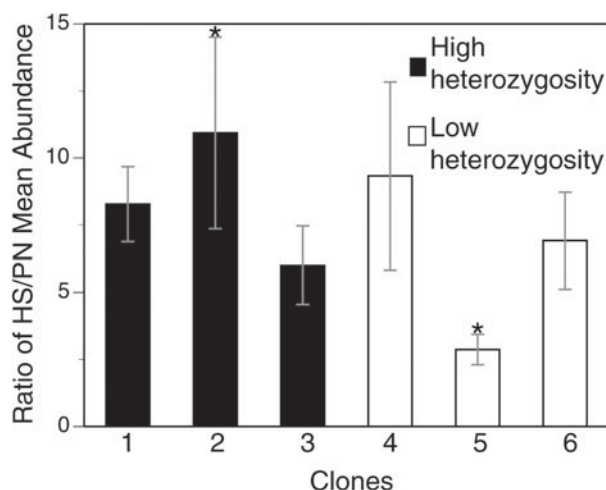


Fig. 2. Mean ( $\pm$ S.E.) ratio of *Heterophoxus stephenseni* (HS) to *Paracalliope novizealandiae* (PN) mean abundance of *Maritrema novaezealandensis* for high and low heterozygosity groups (10 replicates per clone). \* Indicates significant difference between clones. Heterozygosities: Clones 1–3 (56.3%); Clones 4–5 (43.8%); Clone 6 (37.5%).

number of cercariae successfully infected *P. novizealandiae* in the lower heterozygosity than in the higher heterozygosity groups ( $Z=3.948$ ,  $P<0.0001$ ) (Fig. 1). There was no significant difference between the heterozygosity groups for *H. stephenseni* ( $Z=-0.515$ ,  $P=0.607$ ). When examining heterozygosity in relation to the mean abundance ratio of *H. stephenseni* to *P. novizealandiae* across all 10 replicates per clone, we found that all clones had higher infection success in *H. stephenseni* compared to *P. novizealandiae* (Fig. 2). Moreover, the degree

Table 1. Prevalence (mean intensity  $\pm$  S.E.) of *Maritrema novaezealandensis* of each clone in each amphipod species, pooled from 10 replicates

Clone	<i>Heterophoxus stephenseni</i>	<i>Paracalliope novizealandiae</i>
1	91.3 (11.4 $\pm$ 1.2)	63.3 (2.3 $\pm$ 0.2)
2	88.6 (11.3 $\pm$ 1.3)	65.9 (2.2 $\pm$ 0.2)
3	81.3 (4.6 $\pm$ 0.6)	49.5 (1.6 $\pm$ 0.1)
4	81.6 (8.5 $\pm$ 1.4)	58.1 (2.3 $\pm$ 0.2)
5	81.6 (8.0 $\pm$ 1.1)	78.5 (3.2 $\pm$ 0.3)
6	84.8 (11.6 $\pm$ 1.3)	77.4 (2.5 $\pm$ 0.3)

to which the clones preferred *H. stephenseni* varied significantly amongst clones ( $\chi^2=14.73$ , D.F.=5,  $P=0.0116$ ) (Fig. 2). Overall, parasites had a higher prevalence and higher mean intensity in *H. stephenseni* compared to *P. novizealandiae* (Table 1).

#### Environmental refuge experiment

In the absence of sand, *H. stephenseni* had significantly higher infection levels than *P. novizealandiae* ( $Z=7.601$ ,  $P<0.0001$ ) (Fig. 3). However, in the presence of sand there was no significant difference in infection levels between the two amphipod species ( $Z=-1.083$ ,  $P=0.278$ ) (Fig. 3). Infection levels were higher for *H. stephenseni* when sand was absent ( $Z=6.907$ ,  $P<0.0001$ ) and there was no difference in infection levels between the environments for *P. novizealandiae* ( $Z=-0.904$ ,  $P=0.366$ ) (Fig. 3) (Table 2).

#### DISCUSSION

The factors controlling host specificity in parasites remain poorly investigated in parasites of no medical or veterinary concern (Combes, 2001; Poulin and Keeney, 2008). Here, we show that in the marine trematode *Maritrema novaezealandensis* infecting crustacean second intermediate hosts, there are both genetic differences among clones in host preferences, as well as strong influences of environmental conditions on patterns of host use.

#### Genotype experiment

We tested the prediction that parasite clones with higher heterozygosities would have higher infection success (higher fitness) than those with lower heterozygosities. When each host species was examined separately we found the opposite, i.e. parasite clones with lower heterozygosities had significantly higher infection success than those with higher heterozygosities in the amphipod *Paracalliope novizealandiae*. Given that we found significant differences among clones in host preferences, this may suggest that the



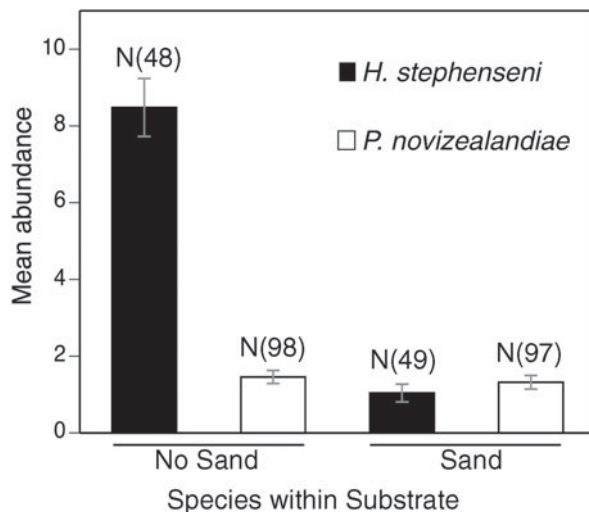


Fig. 3. Mean abundance ( $\pm$  S.E.) of *Maritrema novaezealandensis* in the amphipods *Heterophoxus stephensi* and *Paracalliope novizealandiae* when exposed in an environment with and without sand. In the absence of sand, *H. stephensi* had significantly higher infection levels than *P. novizealandiae*. Infection levels were significantly lower for *H. stephensi* when sand was present.

clones used in this experiment happened to be specialized for *P. novizealandiae* and therefore that homozygotes were at an advantage over the heterozygotes. If clones were specialized for *P. novizealandiae*, then it would make sense that there would be no discernable difference in infectivity when they are exposed to the other amphipod, *Heterophoxus stephensi*. More realistically, finding a negative heterozygosity fitness correlation is not surprising considering that in a recent review by Chapman *et al.* (2009), 34% of the 628 effect sizes were negative. Perhaps cercarial infection success is not the best measure of fitness for this system as it may not be under directional selection. A better measure of clonal fitness could be the number of metacercariae that reach maturity without killing their host.

When we examined the parasite mean abundance ratio of *H. stephensi* to *P. novizealandiae* as a function of heterozygosity, it was clear that *H. stephensi* consistently acquired more parasites than *P. novizealandiae* for each clonal group. It was also evident that host preferences were not standard among all clones, as some had significantly higher ratio values than others. The lower relative preference for *H. stephensi* by clone no. 5, for instance, could be due to some morphological, physiological or behavioural characteristics that consistently reduce exposure to that amphipod relative to *P. novizealandiae*. Whatever the explanation, this is a rare demonstration of genetic variation in host use within a parasite species (see also Downes, 1986; Jaenike and Dombeck, 1998; Paterson, 2005; Detwiler *et al.* 2010). This variation provides the raw material

Table 2. Prevalence (mean intensity  $\pm$  S.E.) of *Maritrema novaezealandensis* in each host species and for each substrate condition, pooled from 10 replicates

Substrate	<i>Heterophoxus stephensi</i>	<i>Paracalliope novizealandiae</i>
Sand	49.0 (2.1 $\pm$ 0.4)	57.7 (2.3 $\pm$ 0.2)
No sand	89.6 (9.5 $\pm$ 0.7)	64.3 (2.3 $\pm$ 0.2)

for selection to generate *M. novaezealandensis* strains specialized for different host species.

Despite these clonal differences in host use, the association between heterozygosity and fitness remains inconclusive. It is possible that the range of heterozygosities chosen for this study (56.25%–37.50%) was not sufficiently wide to see measurable differences in performance. Additionally, more clones could provide the resolution needed to differentiate heterozygosity differences.

#### Environmental experiment

The second experiment compared patterns of host use by *M. novaezealandensis* in the presence and in the absence of sand, to determine if infections of one host species (*H. stephensi*) by the parasite would decrease when a refuge against infection is provided for that host. Clearly, the absence of sand leads to higher infection levels in *H. stephensi* than *P. novizealandiae*. *Heterophoxus stephensi* has a larger surface area and therefore may come into more frequent contact with cercariae compared to *P. novizealandiae*. Additionally, *H. stephensi* may not have adequate deterrent behaviour (evasion) or morphology necessary to prevent cercarial infections when its normal microhabitat is unavailable.

When sand was present *H. stephensi* avoided infection by seeking refuge in it. This left higher numbers of cercariae to potentially infect *P. novizealandiae*, and yet, cercarial infection levels in *P. novizealandiae* were indistinguishable between the sand and sand-free environments. Multiple factors could contribute to *P. novizealandiae*'s relatively low infection levels in the sand habitat. First, *Maritrema novaezealandensis* cercariae exhibit positive geotaxis (A. Koehler, personal observation) whereby they tend to swim at the bottom of the water column along the substrate. The presence of sand may inhibit their movement or create a physical disturbance, as suggested with other larval helminth stages by Thieltges *et al.* (2008). Second, *P. novizealandiae* could be well-adapted for avoiding or deterring cercariae regardless of the substrate. Although the two amphipods used in this study live in overlapping microhabitats, *H. stephensi* spends more time beneath the sediment, allowing for less

exposure to the parasite, while *P. novizealandiae* comes into more direct contact with cercariae, as evidenced by the natural infection prevalences. *Paracalliope novizealandiae* could be under greater natural selection pressure towards increased resistance to the parasite compared to *H. stephenseni*. Therefore *P. novizealandiae*'s avoidance mechanisms (in terms of swimming speed and maneuverability) may be more efficient than those of the naturally less exposed *H. stephenseni* which has a comparatively sluggish movement. Similarly, a local adaptation study by Bryan-Walker *et al.* (2007) demonstrated that when *P. novizealandiae* from parasite-free sites were subjected to *M. novaezealandensis*, they were less likely to resist infection compared to amphipods that had regular exposure to the parasite. This supports the idea that selection pressures from parasites can favour the evolution of resistance or avoidance mechanisms.

In conclusion, we found that (i) there were significant differences among clones in the strength of their preferences for *H. stephenseni*, (ii) there was no clear association between heterozygosity and parasite fitness or host preferences, and (iii) host use by the parasite was clearly affected by the presence of a refugium, which was important enough to offset the intrinsic preferences of the parasite for *H. stephenseni*. The trematode studied here also uses other crustaceans as second intermediate hosts (Koehler and Poulin, 2010), which suggests that overall patterns of host use will involve complex interactions between parasite genotypes and a myriad of environmental factors.

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