

ENVIRONMENTAL AND EVOLUTIONARY DETERMINANTS OF THERMAL TOLERANCE VARIATION IN DIVERGENT PRISTIONCHUS PACIFICUS CLADES

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Abstract

Thermal tolerance is an important adaptive trait that influences ecological distribution and evolutionary divergence in free-living nematodes. Environmental gradients such as altitude may shape thermal exposure and contribute to phenotype differentiation among *Pristionchus pacificus* clades. The present study evaluated the environmental and evolutionary determinants of thermal tolerance variation among divergent *P. pacificus* clades. A quantitative retrospective analysis was conducted using secondary data from 310 *P. pacificus* strains. Thermal tolerance phenotypes, consensus clades, altitude, and environmental temperature were analyzed using descriptive statistics, chi-square test, binary logistic regression, Pearson correlation, and Kruskal–Wallis test. Medium-temperature tolerance was the dominant phenotype, representing 78.39% of strains, followed by high-temperature tolerance at 19.68% and low-temperature tolerance at 1.94%. Consensus clade was significantly associated with thermal tolerance phenotype ($\chi^2 = 135.998$, $df = 8$, $p < 0.001$). Altitude was a significant negative predictor of high-temperature tolerance (OR = 0.9965, $p < 0.001$). Environmental temperature showed a strong negative correlation with altitude ($r = -0.966$, $p < 0.001$), and altitude differed significantly among phenotype groups ($H = 70.056$, $p < 0.001$). These findings indicate that thermal tolerance variation in *P. pacificus* is shaped by both environmental gradients and evolutionary clade structure, supporting the role of altitude-linked thermal conditions in adaptive phenotype distribution.

Keywords: *Pristionchus pacificus*, thermal tolerance, altitude, environmental adaptation, evolutionary clades

1. Introduction

Environmental temperature is an important factor in the ecology of all biological systems, affecting survival, reproduction, physiological functioning and evolutionary adaptations. Thermal changes may modify species distributions, metabolic

activity, development and interaction. The impact of temperature on living systems has become more of a focus recently as environmental instability and change related to climate are changing adaptive responses in terrestrial and aquatic communities. Temperature has also been identified as a key regulator of reproductive and selective processes impacting organismal fitness and population dynamics (García-Roa et al., 2020) under different environmental conditions. Furthermore, the adaptation to environmental stress can happen at various time scales, from short-term physiological changes to long-term changes in populations due to chronic ecological stress (Kristensen et al., 2020). In addition, evidence shows that upper temperature limits ultimately set the limits to life and survival in extreme environments (Asseng et al., 2021).

The nematodes are some of the most ecologically diverse organisms found and have a wide range of habitats - marine, freshwater and terrestrial. They are widely distributed across the environment, have high adaptive flexibility and are evolutionarily diversified along ecological gradients. Nematodes have evolved and adapted a variety of ecological strategies in relation to environmental persistence and adaptation, with habitat transitions playing a role in their evolution (Holterman et al., 2019). Recent studies on free-living nematodes have shown that they play a significant role in ecological monitoring, environmental biology, and evolutionary studies (di Montanara et al., 2022). Among these, *Pristionchus pacificus* has become a useful species for the study of developmental biology, environmental adaptation and evolution. Species have well-documented anatomical and physiological traits that aid investigations of ecological and adaptive responses to environmental variability (Schroeder, 2021).

Thermal tolerance is one of the most important adaptive characteristics to cope with the stressful conditions imposed by the changing temperature. Environmental selection may be sufficient to drive towards the evolution of phenotypic plasticity, which can ensure the functional stability of populations in the face of evolving thermal environments. Experimental approaches have shown that phenotypic plasticity and thermal tolerance evolve differently when exposed to fast and slow temperature changes, suggesting that there is a strong influence of environmental variability on shaping adaptive responses (Schaum et al., 2022). Even the level of phenotypic plasticity can play a role in long-term adaptation by genetic accommodation and assimilation, which can affect evolutionary response to climate-related stress (Kelly, 2019). Thermal stress adaptation in nematodes is a process that relies on a network of physiological and molecular mechanisms involved in survival under high temperatures (Kyriakou et al., 2022). Nematodes also have the ability to sense temperature changes and change their behaviors according to those temperatures, while also sensing context and adjusting their ecological interactions accordingly (Glauser, 2022).

Altitude gradients offer a natural system for exploring ecological adaptation and variation of thermal tolerance. The elevation gradient typically has a decline in temperature and creates different environmental stress on the species represented, which affects their biodiversity patterns, physiological activity, and species composition. Climate change-related disturbances can impact high-elevation ecosystems, potentially changing the interactions and adaptive response of the resident organisms, especially in this context (Seastedt & Oldfather, 2021). Thermal tolerance often varies among organisms that are distributed along gradients in elevation, as temperature is a selective pressure that affects local adaptation. This environmental heterogeneity can then be a factor in the differentiation of phenotypes within geographically localized natural populations.

Genetic divergence and evolutionary lineage structure also play a role in adaptation to environmental stress. Adaptive evolution is based on genetic variation, which allows populations to react in different ways to ecological pressures and environmental conditions. Recent studies in the field of evolution have highlighted the significance of the genetic basis of adaptation and ecological specialization between different populations that are facing different environmental challenges (Bomblies & Peichel, 2022). The evolutionary divergence and ecological differentiation of nematodes may thus be gauged by clade-specific variation in thermal tolerance. A combination of environmental conditions and clade structure could be investigated to better understand the distribution of adaptive phenotypes among genetically distinct lineages.

Most of the previous research has been on physiological reactions to temperature stress, phenotypic plasticity or molecular adaptation in isolated settings. While there are extensive studies on the nature of thermal adaptation in various biological systems, there have been comparatively few studies that attempt to look at the evolutionary clade variation in thermal tolerance phenotypes simultaneously in the face of environmental gradients in free-living nematodes. Specifically, there has been little focus on understanding how environmental variation at different altitudes influences the distribution of phenotypes known to be tolerant of high vs. low temperatures among different clades of the divergent *Pristionchus pacificus* species. There have also been limited efforts to integrate ecological and evolutionary points of view in the analysis of patterns of thermal adaptation from secondary datasets of biological data.

In the present study, we thus examined the environmental and evolutionary factors that underlie the variation in thermal tolerance among divergent *Pristionchus pacificus* clades. The study focused on the distribution of thermal tolerance phenotypes in the strains analyzed, the correlation between the difference in phenotype and the consensus clade, the correlation between environmental temperature and altitude and the differences in thermal tolerance phenotype between the environmental temperature and the presence or absence of the high-temperature tolerance phenotype, and if there were a significant correlation, whether the differences in the presence and absence of the high-temperature tolerance phenotype were predicted by environment and/or the consensus clade. The study aimed to integrate the ecological and evolutionary aspects of adaptive thermal responses into a single analytical approach and to help inform existing knowledge on adaptive thermal responses in free-living nematodes.

2. Methodology

2.1 Research Design

The design of this study was a quantitative retrospective research design of secondary data analysis. The strategy was chosen as it would allow the study of the link between environmental variables, evolutionary clades and thermal tolerance phenotypes in *Pristionchus pacificus*. The present study was directed towards the detection of statistical associations among the distribution of phenotypes, altitude and environmental temperature, based on published biological data.

2.2 Data Source

The data set that was used in the study was derived from an environmental adaptation data set that had been previously generated by Leaver (2022). The data set had ecological data, phenotypic data, and clade data for *Pristionchus pacificus* strains that had been collected from various environments. Thermal tolerance phenotypes, altitude, association with beetle hosts, and environment temperature measurements were among the variables measured.

2.3 Study Variables

Thermal Tolerant phenotype (high temperature tolerant, Htt, medium temperature tolerant, Mtt, and low temperature tolerant, Ltt) was the main outcome variable in this study. Predictor variables were: Consensus clade classification, altitude, and environmental temperature. In descriptive analysis, additional information about ecology, such as beetle host association and strain location, was also explored.

2.4 Data Preparation and Processing

The data cleaning process involved checking the consistency of variables, eliminating duplicate data, transforming numerical data to the correct format, and consolidating categorical variable labels. Records with missing phenotype data were not included in the inferential analysis. To minimize the error of the internal consistency within the observations, the thermal tolerance indicators were validated with the associated binary phenotype variables. The cleaned data were then presented in analytical tables for descriptive and inferential statistics.

2.5 Statistical Analysis

Since these data were summarized with descriptive statistics, frequencies for phenotypes, distribution of consensus clades and characteristics of altitudinal range were summarized. Categorical variables were summarised with frequencies and percentages, and continuous variables with means, medians, standard deviations and ranges. A chi-square test of independence was used to test the association between consensus clade and thermal tolerance phenotype. A binary logistic regression was done to determine if altitude and consensus clade were significant predictors for the occurrence of high temperature tolerance. Odds ratios and 95% confidence intervals were used to provide an estimation of the strength of association. A Pearson correlation analysis was used to determine the correlation between the environmental temperature and altitude. Because the distributions of the altitudes were not normally distributed, Kruskal-Wallis Analysis of Variance for ordinal data was performed between the thermal tolerance phenotypes to compare differences in the variation of temperature with altitude. The p-value of <0.05 was used as the criterion for statistical significance.

3. Results

3.1 Distribution of Thermal Tolerance Phenotypes

Overall, 310 *Pristionchus pacificus* strains were analyzed. The most common phenotype was the medium-temperature-tolerant (Mtt) with 78.39 % of all strains. The high-temperature-tolerant (Htt) strains accounted for 19.68%, while the low-temperature-tolerant (Ltt) strains were relatively uncommon, making up 1.94% of all observations. The majority of the analyzed strains (Mtt phenotype) suggest an adaptive state of intermediate thermal tolerance. Table 1 shows the distribution of the thermal tolerance phenotypes of all the sampled strains. The relative abundance of each thermal tolerance phenotype for the study population is shown in Figure 1.

Table 1. Distribution of thermal tolerance phenotypes among *Pristionchus pacificus* strains

Phenotype	Frequency	Percentage (%)
Mtt	243	78.39
Htt	61	19.68
Ltt	6	1.94

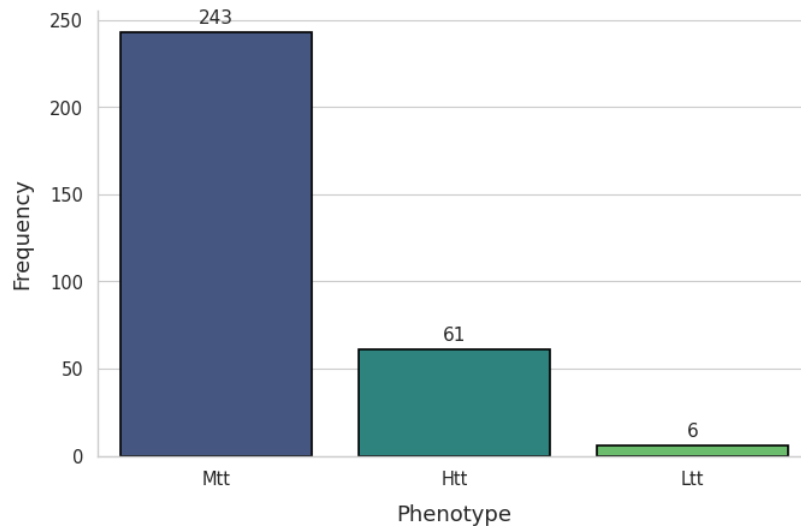


Figure 1. Distribution of thermal tolerance phenotypes among *Pristionchus pacificus* strains

3.2 Distribution of Consensus Clades

Five consensus clades were found throughout the data set. Clade C (37.74%) had the highest proportion of strains, followed by clade D (31.29%). Smaller proportions of the population structure were contributed by the UK, B and A clades. The cladistic distribution observed shows significant genetic variation in the strains of nematodes sampled. The distribution of consensus clades of the analyzed strains is summarized in Table 2.

Table 2. Distribution of consensus clades among *Pristionchus pacificus* strains

Consensus Clade	Frequency	Percentage (%)
C	117	37.74
D	97	31.29
UK	41	13.23
B	28	9.03
A	27	8.71

3.3 Association Between Consensus Clade and Thermal Tolerance Phenotype

The consensus clade was found to be significantly associated with thermal tolerance phenotype ($\chi^2 = 135.998$, $df = 8$, $p < 0.001$). Different phenotypical patterns were found within the clades. All the identified Ltt strains were in clade B, while clade A had a relatively high proportion of Htt strains. The Mtt phenotype was mainly associated with clades C and D. The results suggest that all these traits are not equally distributed among evolutionary lineages and are subjected to thermal tolerance. Figure 2 illustrates the association between consensus clades and thermal tolerance phenotype.

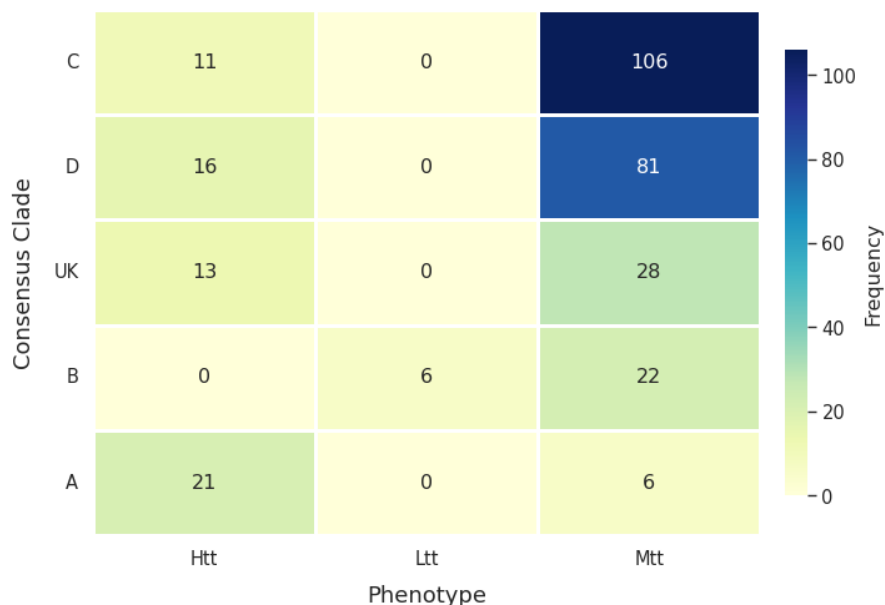


Figure 2. Heatmap showing the association between consensus clade and thermal tolerance phenotype

3.4 Logistic Regression Analysis of High-Temperature Tolerance

Altitude and consensus clade were tested for their significant predictor of the presence of high-temperature tolerance using binary logistic regression analysis. A significant negative association was attained between Htt occurrence and altitude (OR = 0.9965, $p < 0.001$), showing a decrease in Htt tolerance with increasing altitude. The odds of having the Htt phenotype were significantly lower for clades C and D than for the reference clade. There was no statistically significant association with Htt occurrence that was found by Clade UK. Table 3 shows the logistic regression model used to assess the predictors of tolerance to high temperature.

Table 3. Logistic regression analysis predicting high-temperature tolerance phenotype

Variable	Coefficient	Odds Ratio	P-value	95% CI Lower	95% CI Upper
Constant	1.5943	4.9250	0.001181	1.8793	12.9066
Altitude	-0.0035	0.9965	0.000018	0.9949	0.9981
Clade C	-1.7018	0.1823	0.009344	0.0505	0.6580
Clade D	-1.5028	0.2225	0.016819	0.0649	0.7628
Clade UK	-0.0737	0.9289	0.920189	0.2196	3.9293

3.5 Relationship Between Altitude and Environmental Temperature

The correlation of environmental temperature with altitude was strong and negative ($r = -0.966$, $p < 0.001$). The environmental temperature was significantly higher in lower elevation areas and significantly lower in higher elevation areas. This inverse relationship was very strong, implying that altitude is a significant environmental factor that affects the local thermal conditions at the sampled habitats. The regression relationship between the environmental temperature and altitude is shown in Figure 3.

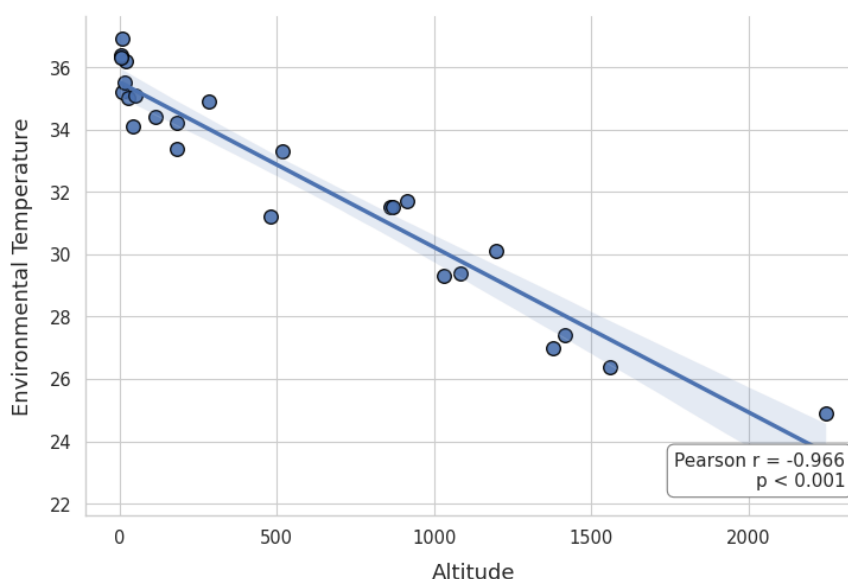


Figure 3. Relationship between altitude and environmental temperature across sampling locations

3.6 Altitude Variation Among Thermal Tolerance Phenotypes

Thermal tolerance phenotypes were significantly different with respect to their altitude distributions (Kruskal–Wallis $H = 70.056$, $p < 0.001$). The htt strains were mostly related to low altitude sites, with a mean of 331.80 m. By contrast, the Mtt strains had a wider distribution of altitude with an average of 866.32 m. This Ltt phenotype was found only in high altitudes, with the maximum mean altitude (2206.33 m). The results of the altitude variation across the thermal tolerance phenotypes are summarized in Table 4.

Table 4. Altitude distribution across thermal tolerance phenotypes

Phenotype	Count	Mean	Median	Standard Deviation	Minimum	Maximum
Htt	61	331.80	339.0	281.51	17	886
Ltt	6	2206.33	2146.0	93.47	2146	2327
Mtt	243	866.32	694.0	567.87	17	2327

4. Discussion

The variation of thermal tolerances for *Pristionchus pacificus* is clearly an ecological and evolutionary pattern. Intermediate thermal tolerance might be the most prevalent adaptive state of the analyzed strains since the medium-temperature-tolerant phenotype is predominant. This distribution may suggest that the majority of the strains can be acclimatized to moderate environmental conditions, but not to harsh thermal environments. Extreme thermal phenotypes seem to be less common than expected, and the low-temperature-tolerant strains are even less so, indicating that extreme thermal phenotypes are more limited and possibly related to specific ecological niches. The consensus clade was strongly linked to thermal tolerance phenotype, suggesting that thermal tolerance was not randomly distributed among the evolutionary lineages. Clade A had a larger percentage of the strains with a high temperature tolerance, while clade B had all the strains with a low temperature tolerance. The medium-temperature-tolerant strains were the predominant representatives of clades C and D. The patterns indicate that the evolutionary background may be an important factor in differentiation of phenotypes, and that some clades seem to have a greater affinity to certain thermal responses than others. Environmental factors influencing thermal tolerance seemed to be important, and altitude was one of them. Low-temperature-tolerant strains were found primarily in the high elevations, whereas high-temperature-tolerant strains were restricted to low elevations. The strong negative correlation of environmental temperature with elevation is consistent with the hypothesis that elevation is an ecological gradient that influences thermal exposure. Logistic regression also showed that the probability of high-temperature tolerance decreased with increasing altitude and that the clade had a significant impact on the probability of expressing the high-temperature-tolerant phenotype.

The observed correlation between the thermal conditions and nematode phenotype is consistent with experimental evidence that free-living nematodes' population growth and body-size patterns can be affected by temperature (Majdi et al., 2019). The observed distribution of high-temperature-tolerant strains in lower altitudes, warmer climates is also consistent with other studies that indicate the performance of helminths can be influenced by local climatic conditions (Aleuy et al., 2023). Clade-associated variation observed in the present analysis is consistent with the findings of other studies that found phenotypic differences and fitness trade-offs along environmental gradients are associated with thermal adaptation (Sherpa et al., 2022). Likewise, the high altitude-temperature gradient is indicative of broader biogeographic patterns of thermal tolerance variation along elevation and latitude gradients, consistent with environmental filtering along spatial gradients (Sunday et al., 2019).

Additional evidence supporting the biological relevance of temperature as a selective pressure is that cellular and organismal traits in ectothermic animals can be influenced by thermal conditions (Friesen et al. 2022). Research also indicates that dauer-related traits are responsible for nematode adaptation to unfavorable ecological conditions, thus supporting the role of evolutionary and developmental adaptation in nematodes (Vlaar et al., 2021). Additionally, based on comparative transcriptomic data spanning nematode life cycles, there is evidence that some genes are expressed in the same manner across developmental stages and lineages while others are not (Lu et al., 2020).

The results indicate that both environmental gradients and evolutionary background influence the thermal tolerance of *P. pacificus*. The joint effect of altitude and environmental temperature, together with the clade structure, is useful to consider when trying to elucidate the interaction between ecological conditions and lineage history on adaptive phenotype distribution. The results suggest that a lower elevation site might support the high-temperature-tolerant strains, and a higher elevation site might support the cold-associated or low-temperature-tolerant phenotypes as seen from an ecological perspective. The thermal phenotypic variation across clades indicates, from an evolutionary point of view, that adaptive divergence might be a factor for ecological specialization in *P. pacificus*. These results have implications for the general discussion of adaptation to climate change, particularly in the case of organisms living in a heterogeneous environment.

A few limitations should be acknowledged. A secondary study was conducted, and thus only the variables that were available in the secondary data set were analyzed. Apart from altitude and temperature, other habitat factors, including microclimate, humidity and soil conditions, were not used to consider the habitat for the interpretation. This may also affect the conclusions that can be drawn for that phenotype due to the low count of low-temperature-tolerant strains.

Experimental thermal assays, combined with genomic studies and ecological data, are needed in future to elucidate the mechanism of thermal tolerance in each clade. A larger sample of additional habitats and environmental gradients would also provide a more comprehensive understanding of the response of *P. pacificus* to a changing thermal gradient.

5. Conclusion

The variation in thermal tolerance of *Pristionchus pacificus* seems to be influenced both by an environmental gradient and by evolutionary clade structure. Medium-temperature tolerance was the most predominant phenotype, with more narrowly distributed ecological distributions for high- and low-temperature tolerance phenotypes. Thermal tolerance is not distributed at random within evolutionary lineages, as there is a significant association between consensus clade and phenotype. Altitude was also found to be an important ecological factor; high-temperature-tolerant strains were found primarily at lower altitudes, while low-temperature-tolerant strains were found primarily at higher altitudes. The negative correlation between altitude and environmental temperature is also reflected in the strong negative correlation between this thermal gradient and the distribution of adaptive phenotypes. These findings indicate that environmental temperature and clade-associated divergence play a role in thermal adaptation of *P. pacificus*. The results offer valuable insights into the ecological specialization of free-living nematodes and will facilitate further research into this question through experimental thermal assays, genomic analysis, and environmental sampling.

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