Main question:

Does the temperature history of rotifers affect competition with protists?

Sub-questions:

1. Did the evolution period lead to changes to rotifers’ response to temperature?
2. Do rotifers have a competitive response to the protist?
3. Do rotifers have a competitive effect on the protist?

Following are answers to the above questions, observed in two growth parameters: maximum population growth rate (hereafter: growth rate, *r*) and carrying capacity (*K*).

1. Population growth rate (*r*)

Table 1a. Rotifers: three-factor ANOVA of ln-transformed *r*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Predictor | df | SS | MS | F | p |  |
| evolved temp | 1 | 0.2901 | 0.2901 | 1.4811 | 0.22757 |  |
| current temp | 1 | 0.9049 | 0.9049 | 4.6207 | 0.03495 | \* |
| competition | 1 | 3.6387 | 3.6387 | 18.5796 | <0.001 | \*\*\* |
| evol:current | 1 | 0.3074 | 0.3074 | 1.5698 | 0.21429 |  |
| evol:comp | 1 | 0.0382 | 0.0382 | 0.1951 | 0.66004 |  |
| current:comp | 1 | 1.3423 | 1.3423 | 6.8539 | 0.01078 | \* |
| evol:current:comp | 1 | 0.0015 | 0.0015 | 0.0075 | 0.93111 |  |
| residuals | 72 | 14.1007 | 0.1958 |  |  |  |

Table 1b. Protists: two-factor ANOVA of ln-transformed *r*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Predictor | df | SS | MS | F | p |  |
| current temp | 1 | 4.5647 | 4.5647 | 21.3398 | <0.001 | \*\*\* |
| competition | 2 | 0.3627 | 0.1813 | 0.8478 | 0.43398 |  |
| current:comp | 2 | 1.9391 | 0.9696 | 4.5327 | 0.01515 | \* |
| residuals | 54 | 11.5508 | 0.2139 |  |  |  |

i. Evolution of temperature response in rotifers (*r*)

The growth rate of rotifers did not differ between the two evolutionary histories of temperature (F1,72 = 1.481, p = 0.228; Table 1a).

ii. Rotifer competitive response (*r*)

Competition did affect rotifer growth rate, but was dependent on current temperature (F1,72 = 6.854, p = 0.011; Table 1a). Growth rate was 60% higher (back-transformed data) in the absence of a competitor at 30C (estimate = 0.686 ± 0.14 SE, t72 = 4.899, p < 0.001) but competition had no effect on growth rate at 25C (estimate = 0.167 ± 0.14 SE, t72 = 1.197, p = 0.235).

iii. Rotifer competitive effect (*r* of protists)

Competition affected the growth rate of protists, but the effect was dependent on current temperature (F1,54 = 4.533, p = 0.015; Table 1b). Post-hoc comparisons on the levels of competition (no competition, competition with rotifers evolved at 25C, and competition with rotifers evolved at 30C) show protist growth rate was further dependent on the evolutionary history of their rotifer competitors. At a current temperature of 30C, protist growth rate was 30% lower (back-transformed data) in the presence of rotifers, but only if the rotifers evolved at 30C (estimate = 0.577 ± 0.207 SE, t54 = 2.789, p = 0.0196). At the same current temperature of 30C, rotifers with an evolutionary history of 25C did not have an effect on protist growth rate (estimate = 0.404 ± 0.207 SE, t54 = 1.953, p = 0.1339). Protist growth rate was unaffected by the presence of competitors when the current temperature was 25C (all p > 0.05, Sup. Table 1).

2. Carrying capacity (*K*)

Table 2a. Rotifers: three-factor ANOVA of *K*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Predictor | df | SS | MS | F | p |  |
| evolved temp | 1 | 599.8 | 599.8 | 4.2537 | 0.04277 | \* |
| current temp | 1 | 522.1 | 522.1 | 3.7028 | 0.05827 | . |
| competition | 1 | 6211.0 | 6211.0 | 44.0512 | <0.001 | \*\*\* |
| evol:current | 1 | 20.4 | 20.4 | 0.1450 | 0.70449 |  |
| evol:comp | 1 | 144.1 | 144.1 | 1.0223 | 0.31537 |  |
| current:comp | 1 | 20.3 | 20.3 | 0.1442 | 0.70525 |  |
| evol:current:comp | 1 | 1.5 | 1.5 | 0.0106 | 0.91811 |  |
| residuals | 72 | 10151.6 | 141.0 |  |  |  |

Table 2b. Protists: two-factor ANOVA of *K*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Predictor | df | SS | MS | F | p |  |
| current temp | 1 | 11129 | 11129 | 0.4755 | 0.49341 |  |
| competition | 2 | 327742 | 163871 | 7.0021 | 0.00198 | \*\* |
| current:comp | 2 | 802975 | 401488 | 17.1554 | <0.001 | \*\*\* |
| residuals | 54 | 1263761 | 23403 |  |  |  |

i. Evolution of temperature response in rotifers (*K*)

Evolved temperature affected the carrying capacity of rotifers independent of current temperature and the presence of a competitor (F1,72 = 4.254, p = 0.043; Table 2a). The carrying capacity of rotifers that evolved at 25C was 9% higher than those that evolved at 30C (estimate = 5.48 ± 2.66, t72 = 2.062, p = 0.0428).

ii. Rotifer competitive response (*K*)

Competition affected the carrying capacity of rotifers independent of current temperature and evolved temperature (F1,72 = 44.051, p = <0.001; Table 2a). Rotifer carrying capacity was 26% higher in the absence of the protist competitor (estimate = 17.6 ± 2.66, t72 = 6.637, p = <0.001).

iii. Rotifer competitive effect (*K* of protists)

Competition affected the carrying capacity of protists, but the effect was dependent on current temperature (F1,54 = 17.1554, p = <0.001; Table 2b). Protist carrying capacity was 36% lower in the presence of rotifer competitors, but only at a current temperature of 25C (estimate = -395.9 ± 59.2, t54 = -6.681, p = <0.001). The carrying capacity of protists was unaffected by the evolutionary history of their rotifer competitors (p = >0.05).

Supplemental Table 1. EMMs from a two-factor ANOVA of ln-transformed protist *r*. No competition = “no comp", rotifers evolved at 25C = “rotif25”, rotifers evolved at 30C = “rotif30”. All *p-*values are Tukey’s HSD-adjusted.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Current temp | | Contrast | Estimate | | SE | Df | t | p |  |
| 25C | noComp – rotif25 | | -0.326 | 0.207 | 54 | -1.575 | 0.2653 |  |
| noComp – rotif30 | | -0.215 | 0.207 | 54 | -1.039 | 0.5555 |  |
| rotif25 – rotif30 | | 0.111 | 0.207 | 54 | 0.535 | 0.8544 |  |
| 30C | noComp – rotif25 | | 0.404 | 0.207 | 54 | 1.953 | 0.1339 |  |
| noComp – rotif30 | | 0.577 | 0.207 | 54 | 2.789 | 0.0196 | \* |
| rotif25 – rotif30 | | 0.173 | 0.207 | 54 | 0.836 | 0.6823 |  |