Objective/Hypotheses/Methods

Example:

Objectives.

The epigenome is most susceptible to environmentally-induced changes at the prenatal stage, when epigenetic marks are re-established following erasure after fertilization [15, 16]. The proposed study will investigate the impact of exposure to high temperature at early versus late stages of embryonic development on C. scorpioides' nymphal survival, developmental time, and adult morphological and reproductive traits. ncRNAs will be extracted from the testicular and ovarian tissues of experimental individuals, and frozen for subsequent library construction and RNA sequencing. The aim of this project is to increase our understanding of the life history stage at which tropical ectotherms are most vulnerable to climate warming.

Research hypothesis.

This study will test the hypothesis that exposure to high temperature during embryonic development has a negative impact on this neotropical pseudoscorpion. Since epigenetic reprogramming occurs soon after fertilization, it is predicted that the detrimental effects of increased temperature will be more severe in individuals that experience high temperature as early-stage embryos, compared to those that are exposed later in embryonic development. Early embryonic exposure is also predicted to cause greater disruption to small ncRNA expression profiles than late exposure.

Plans for research.

Temperature variation during embryonic development. Matings will be carried out in three blocks with 27 pairs of unrelated males and females per block. The females will be randomly assigned to one of three diurnally-fluctuating temperature treatments: an early exposure (E) treatment; a late exposure (L) treatment, and a control (C) treatment. Females in the C treatment will be maintained throughout gestation in an incubator programmed for an average temperature of 27.3°C, the average current temperature in C. scorpioides' natural habitat. In the E treatment, females will be transferred to an identical incubator programmed for an average temperature of 29.3°C (+ 2°C) during embryonic days 1-5 (E1-5) and then returned to the control incubator. L females will be held at the control temperature through E5, and then switched to the higher temperature incubator for E6-10. On Day 5 of embryonic development, the brood sacs of all gravid females will be photographed at high magnification, and the number of embryos counted. The reproductive status of females will be monitored, cases of spontaneous abortion will be recorded, and the number of protonymphs (first-stage nymphs) born to females that successfully carry broods to term will be counted in order to document survivorship.

Rearing nymphs to adulthood. A subset of 20 protonymphs from each female will be reared to the adult stage in individual vials at the control temperature. Development time from birth to adulthood will be recorded, and, for vials not yielding an adult within 60 days after birth, the nymph will be scored as not having survived.

Adult traits. To assess temperature effects on morphological traits, male and female adult offspring from the E, L and C treatments will be photographed at high magnification, and five traits of the pedipalps (appendages used in prey capture and male combat) and cephalothorax will be measured. To determine effects on reproductive traits, male offspring from the three treatments will be mated to non-experimental females and sperm packets will be collected to quantify the number and viability of sperm. Female offspring from each treatment will be mated to non-experimental males. The number of early-stage embryos the females produce will provide a measure of their fertility, and their reproductive success will be quantified by counting the number of nymphs born.