

# **ESTIMATING POTENTIAL FOR ADAPTATION OF CORALS TO CLIMATE WARMING**

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**CERTIFICATE OF AUTHORSHIP/ORIGINALITY**

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Nikolaus B. M. Császár

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## ABSTRACT

Climate models predict rapidly warming oceans throughout the 21<sup>st</sup> century along with increased mortalities in reef-building coral-algal symbioses. Yet the ability of corals to adapt genetically in an evolutionary sense to a warmer climate is unknown. The adaptive potential of corals can be approximated by the extent to which variation in thermal tolerance is caused by genetic factors (i.e. by the broad-sense heritability,  $H^2$ ). This thesis investigated  $H^2$  in a total of eleven thermal tolerance traits from two populations of the reef-building coral species *Acropora millepora* in the central Great Barrier Reef, Australia. The first population that was investigated associates with thermo-tolerant algal symbionts of the genus *Symbiodinium* (clade D), and came from Magnetic Island (MI), while the second population from Orpheus Island (OI) associates with the intermediately tolerant *Symbiodinium* type C2. Traits investigated were characteristic of the coral host, the algal symbiont, and the holobiont (whole symbiosis).

The present thesis revealed extensive genetic variation in algal symbiont traits, which, together with short generation times, allows for rapid symbiont adaptation to climate warming. A significant adaptive potential was also found for coral colony growth rates, defined here as a holobiont trait. This is in stark contrast to the coral host, which did not display heritability for the majority of the traits investigated for either population. The coral host with its long generation time has therefore only a low potential to adapt to rapidly warming oceans.

Five of the six thermal tolerance traits yielded significant heritabilities in each of the two symbiont types. In clade D symbionts from MI, the adaptive potential was given for the maximum quantum yield of photosystem II, Fv/Fm, one of the most commonly studied stress parameters in coral biology which indicates the overall health condition of photosystems. The one trait that did not yield a significant heritability in D symbionts was non-photochemical quenching ( $\Phi$ NPQ) of excess excitation energy. The trait  $\Phi$ NPQ can be considered as a switch for xanthophyll cycling, a mechanism that protects photosystems through conversion of the pigment diadinoxanthin (DD) into diatoxanthin (DT).

However, D symbionts diverted 50 % of the incoming light energy for the initiation of the xanthophyll cycle (i.e. via  $\Phi$ NPQ), and the xanthophyll cycle mechanism itself showed significant heritability in either symbiont type. Both symbiont types also displayed significant heritability for another measure of photoprotection, the ability to regulate the pool size of photoprotective xanthophyll pigments (XP) relative to total light-harvesting pigments (LH). Although Fv/Fm did not yield a significant heritability in C2 symbionts from OI, both symbiont types again showed heritability for the effective quantum yield of photosystem II ( $\Phi$ PSII), and for unregulated energy dissipation ( $\Phi$ NO).

For traits reflecting the function of the coral host, messenger RNA (mRNA) expression levels of four fundamental genes involved in the oxidative stress response were investigated. These genes code for cellular defences which regulate cellular iron homeostasis (i.e. Ferritin), repair denatured proteins (i.e. the heat shock protein Hsp70), detoxify harmful oxygen radicals (i.e. the mitochondrial enzyme manganese superoxide dismutase MnSOD), and might be involved in the dysfunction of coral cell-adhesion proteins during bleaching via a remodelling of surface receptors in the extra-cellular matrix (i.e. a zinc-metalloprotease,  $\text{Zn}^{2+}$ -met). Each coral host population, however, showed heritability for expression of just one of those four genes (i.e. MnSOD in the MI population, and  $\text{Zn}^{2+}$ -met in the OI population), therefore displaying only a limited capacity for evolution of thermal tolerance.

Holobiont growth showed a significant heritability in both coral-algal populations, thus providing the basis for evolutionary adaptation. In the long term, however, this trait might be impaired by ocean acidification, which has a negative impact on coral calcification and, therefore, on holobiont growth rates.

In summary, algal symbionts have short generation times and considerable genetic variation in functional traits, thus allowing for rapid adaptation to higher temperatures. However, adaptive response estimates based on low heritabilities in coral host traits along with the coral's mainly sexual reproduction and long generation time raise concerns about the timely adaptation of the holobiont in the face of rapid climate warming.

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## LIST OF ABBREVIATIONS

AIMS	Australian Institute of Marine Science
ANOVA	Analysis of Variance
cDNA	Complementary DNA
DD	Diadinoxanthin (xanthophyll pigment, epoxidised)
DT	Diatoxanthin (photoprotective xanthophyll pigment, de-epoxidised)
DT/(DD+DT)	Xanthophyll cycling activity expressed as molar pigment ratio
ECM	Extra cellular matrix
F	Minimum light-adapted fluorescence
F <sub>0</sub>	Minimum dark-adapted fluorescence
F <sub>m</sub>	Maximum dark-adapted fluorescence
F <sub>m</sub> '	Maximum light-adapted fluorescence
F <sub>v</sub>	variable fluorescence yield (=F <sub>m</sub> -F <sub>0</sub> )
F <sub>v</sub> /F <sub>m</sub>	Maximum quantum yield of Photosystem II
GBR	Great Barrier Reef
G x E	Genotype by environment
GLM	General Linear Model
GOI	Gene of interest
$h^2$	Heritability in the narrow sense
$H^2$	Heritability in the broad sense
Hsp70	Heat shock protein 70
ICG	Internal control gene
I-PAM	Imaging PAM
ITS1	Internal transcribed spacer 1
LED	Light emitting diode
LH	Light-harvesting pigments (chlorophyll <i>a</i> , chlorophyll <i>c2</i> , peridinin, DD, DT)
MI	Magnetic Island
MMP	Matrix metalloprotease
MnSOD	Manganese superoxide dismutase
mRNA	Messenger RNA

OI	Orpheus Island
PAM	Pulse amplitude modulated
PCR	Polymerase chain reaction
PSII	Photosystem II
qP	Photochemical quenching
qN	Non-photochemical quenching
$\Phi$ PSII	Effective quantum yield of PSII
$\Phi$ NPQ	Regulated non-photochemical energy dissipation (photoprotective)
$\Phi$ NO	Unregulated energy dissipation (not photoprotective)
qRT-PCR	Quantitative real-time (or reverse transcription) PCR
<i>R</i>	Response to selection
ROS	Reactive oxygen species
<i>S</i>	Selection differential (strength of selection)
SOD	Superoxide dismutase
SSCP	Single strand conformation polymorphism
SST	Sea surface temperature
$T_0$	Time-point before the temperature ramp
$V_A$	Additive genetic variation
$V_D$	Dominance deviation
$V_E$	Environmental variation
$V_G$	Genetic variation
$V_I$	Interaction deviation
$V_P$	Phenotypic variation
XP	Xanthophyll pigments (DD and DT)
XP/(LH+XP)	Molar ratio of xanthophyll to total light-harvesting (including xanthophyll) pigments
Zn <sup>2+</sup> -met	Zinc-metalloprotease

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**Table 3.1** Mean levels ( $\bar{x}$ ) and standard errors ( $\pm$  SE) of antioxidant and stress-gene up-regulation in the bleached-(n=79) relative to the non-bleached (n=79) coral samples; given in percent (%) averaged over 20 colonies. Up-regulation was significant for each of the four genes ( $p \leq 0.01$ ,  $df=78$ , two-tailed  $t$ -test). Correlation coefficients ( $r$ ) for the direction of gene expression change between the four genes across the bleached samples (n=79) are given. Significant positive correlations are indicated with \* ( $p < 0.05$ ), highly significant with \*\* ( $p < 0.01$ ).

**Table 4.1** Overview of heritability and mean changes in thermal tolerance traits under experimental temperatures in a population of *Acropora millepora* from Magnetic Island and its clade D symbionts. Heritability ( $H^2$ , its standard error (SE), and its significance

at  $p < 0.05$ ) and mean percent changes in phenotypic traits (%  $\Delta$ , where - means reduction and + increase ( $\pm$  SE)) are shown. Changes in symbiont pigment ratios are given as factor change. Total sample sizes for the different temperature treatments ranged from  $n=29-40$  for 27 °C, to  $n=80$  for 31 °C and  $n=79-80$  for 32 °C. Temperature treatments for which data were not obtained are indicated as not available (n. a.). Statistical results for heritability are from ANOVA, which were considered significant at  $p < 0.05$ , and for mean changes from two-tailed  $t$ -tests (at  $p < 0.05$ ). Significant heritabilities are indicated bold and with asterisks (\*), and significant mean changes with asterisks. Mean changes in trait values that were obtained after 7 days of exposure to experimental temperatures (as opposed to 9 days) are signified by <sup>a</sup>.

**Table 4.2** Comparison of molar pigment ratios and total pigment concentrations ( $\mu\text{g}$  pigment g coral wet weight<sup>-1</sup>) between initial conditions ( $T_0$ ; 27 °C) and after exposure to 32 °C. The factor changes of ratios and concentrations between the two treatments are also shown. DD – diadinoxanthin, DT – diatoxanthin, XP – xanthophylls, LH –light-harvesting pigments, Chl – chlorophyll.

**Table 5.1** Overview of heritability and mean changes in thermal tolerance traits under experimental temperatures in a population of *Acropora millepora* from Orpheus Island and its type C2 symbionts. Heritability ( $H^2$ , its standard error (SE), and its significance at  $p < 0.05$ ) and mean percent changes in phenotypic traits (%  $\Delta$ , where - means reduction and + increase ( $\pm$  SE)) are shown. Changes in symbiont pigment ratios are given as factor change. Total sample sizes were  $n=80$  for both 27 and 31 °C, and  $n=72$  for 32 °C. Temperature treatments for which data were not obtained are indicated as not available (n. a.). Statistical results for heritability are from ANOVA, which were considered significant at  $p < 0.05$ , and for mean changes from two-tailed  $t$ -tests (at  $p < 0.05$ ). Significant heritabilities are indicated bold and with asterisks (\*), and significant mean changes with asterisks.

**Table 5.2** Comparison of molar pigment ratios and total pigment concentrations ( $\mu\text{g}$  pigment g coral wet weight<sup>-1</sup>) between initial conditions ( $T_0$ ; 27 °C) and after exposure to 32 °C. Changes in ratios and concentrations between the two treatments are shown as factor changes. DD – diadinoxanthin, DT – diatoxanthin, XP – xanthophylls, LH – light-harvesting pigments, Chl – chlorophyll.

**Table 5.3** Overview of heritability and mean changes in thermal tolerance traits under bleaching conditions (32 °C). Heritabilities and mean percent changes (- means reduction and + increase; ( $\pm$  SE)) in the investigated traits in the Orpheus Island (OI) and the Magnetic Island (MI) population of *Acropora millepora*, and their respective type C2 and clade D symbionts. Mean changes in symbiont pigment ratios are given as factor change. Statistical results for heritability are from ANOVA, which were considered significant at  $p < 0.05$ , and for mean changes from two-tailed  $t$ -tests (at  $p < 0.05$ ). Significant heritabilities are indicated bold with asterisks (\*), and mean changes with asterisks. Mean changes in trait values that are compared for seven days of exposure to bleaching conditions (as opposed to nine days) are signified by a.

**Table 5.4** Overview of energy dissipation pathways in both C2 symbionts from Orpheus Island (OI) and D symbionts from Magnetic Island (MI) at the beginning of the experiment (day -5) and after exposure to 32 °C for 7 days (given as percent).  $\Phi$ PSII – effective quantum yield of PSII,  $\Phi$ NPQ – regulated non-photochemical quenching,  $\Phi$ NO – unregulated non-photochemical quenching.

**Appendix B 1 ANOVA table:** Maximum dark-adapted fluorescence yield ( $F_v/F_m$ ) and coral (holobiont) growth.

**Appendix B 2 ANOVA table:** Light-adapted fluorescence yields ( $\Phi$ PSII,  $\Phi$ NPQ, and  $\Phi$ NO).

**Appendix B 3 ANOVA table:** Symbiont pigment ratios ( $DT/(DD+DT)$  and  $XP/(LH+XP)$ ).

**Appendix B 4 ANOVA table:** Coral host gene expression.

**Appendix B 5:** Genotype by environment (G x E) interaction for  $F_v/F_m$  and  $\Phi$ PSII.

**Appendix B 6:** Genotype by environment (G x E) interaction for  $\Phi$ NPQ and  $\Phi$ NO.

**Appendix B 7:** Genotype by environment (G x E) interaction for coral (holobiont) growth.

**Appendix C 1 ANOVA table:** Maximum dark-adapted fluorescence yield ( $F_v/F_m$ ) and coral (holobiont) growth.

**Appendix C 2 ANOVA table:** Light-adapted fluorescence yields ( $\Phi_{PSII}$ ,  $\Phi_{NPQ}$ , and  $\Phi_{NO}$ ).

**Appendix C 3 ANOVA table:** Symbiont pigment ratios ( $DT/(DD+DT)$  and  $XP/(LH+XP)$ ).

**Appendix C 4 ANOVA table:** Coral host gene expression.

**Appendix C 5:** Genotype by environment (G x E) interaction for  $F_v/F_m$  and  $\Phi_{PSII}$ .

**Appendix C 6:** Genotype by environment (G x E) interaction for  $\Phi_{NPQ}$  and  $\Phi_{NO}$ .

**Appendix C 7:** Genotype by environment (G x E) interaction for coral (holobiont) growth.