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SHORT COMMUNICATION

Recent expansion of heat-activated retrotransposons in the coral symbiont *Symbiodinium microadriaticum*

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Rising sea surface temperature is the main cause of global coral reef decline. Abnormally high temperatures trigger the breakdown of the symbiotic association between corals and their photosynthetic symbionts in the genus *Symbiodinium*. Higher genetic variation resulting from shorter generation times has previously been proposed to provide increased adaptability to *Symbiodinium* compared to the host. Retrotransposition is a significant source of genetic variation in eukaryotes and some transposable elements are specifically expressed under adverse environmental conditions. We present transcriptomic and phylogenetic evidence for the existence of heat stress-activated Ty1-*copia*-type LTR retrotransposons in the coral symbiont *Symbiodinium microadriaticum*. Genome-wide analyses of emergence patterns of these elements further indicate recent expansion events in the genome of *S. microadriaticum*. Our findings suggest that acute temperature increases can activate specific retrotransposons in the *Symbiodinium* genome with potential impacts on the rate of retrotransposition and the generation of genetic variation under heat stress.
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Coral reefs are notable for being one of the world's most productive and diverse ecosystems despite flourishing in highly oligotrophic environments (de Goeij *et al*., 2013). This paradox exists because of the endosymbiotic relationship between corals and unicellular algae of the genus *Symbiodinium*, whereby corals provide nutrients and shelter to the algae in exchange for energy in the form of photosynthetically fixed carbon (Muscatine and Porter, 1977). In the past few decades, most coral ecosystems around the world have been gradually declining, a process that has been accelerated by the warming of the oceans (Ainsworth *et al*., 2016). The coral *Symbiodinium* symbiosis is notably sensitive to increases in seawater temperature, which can lead to widespread expulsion of the symbiont and eventual starvation of the coral hosts if the symbiotic relationship is not restored (Hoegh-Guldberg and Smith, 1989). However, the presence of thermotolerant corals, such as those in the Red Sea (Fine *et al*., 2013), strongly suggests that there is room for adaptation to higher ocean temperatures. One component that determines coral thermotolerance is the presence of *Symbiodinium* strains that are more resistant to heat stress (Howells *et al*., 2012). Given the shorter generation times of *Symbiodinium* relative to their coral hosts, it has been speculated that the higher rate at which genetic variation increases in *Symbiodinium* could play a major role in the evolution of coral–algal symbiosis with greater thermotolerance (Csaszar *et al*., 2010; van Oppen *et al*., 2017).

One way in which rapid and environmentally triggered genomic variation could be generated is via retrotransposons, whose capability to replicate independently of the host genome makes them particularly interesting as one of the causal agents of natural insertional mutagenesis (Kazazian, 2004). While most retrotransposons are transcriptionally silenced by the host genome, several lines of evidence suggest that these genetic elements can be activated under certain stress conditions such as wounding (Takeda *et al*., 1998) or UV irradiation (Ramallo *et al*., 2008).

A specific class of retrotransposons, defined by the presence of flanking long terminal repeats (LTR), is notable for being transcriptionally activated by a range of stress conditions in fungi (Anaya and Roncero, 1996), metazoans (Faure *et al*., 1996) and higher plants (Ito *et al*., 2011). An example of this is ONSEN, a Ty1-*copia*-type LTR retrotransposon in *Arabidopsis thaliana* that was shown to be activated by elevated temperatures and capable of generating *A. thaliana* lines resistant to abscisic acid, thereby linking heat stress with the generation of novel

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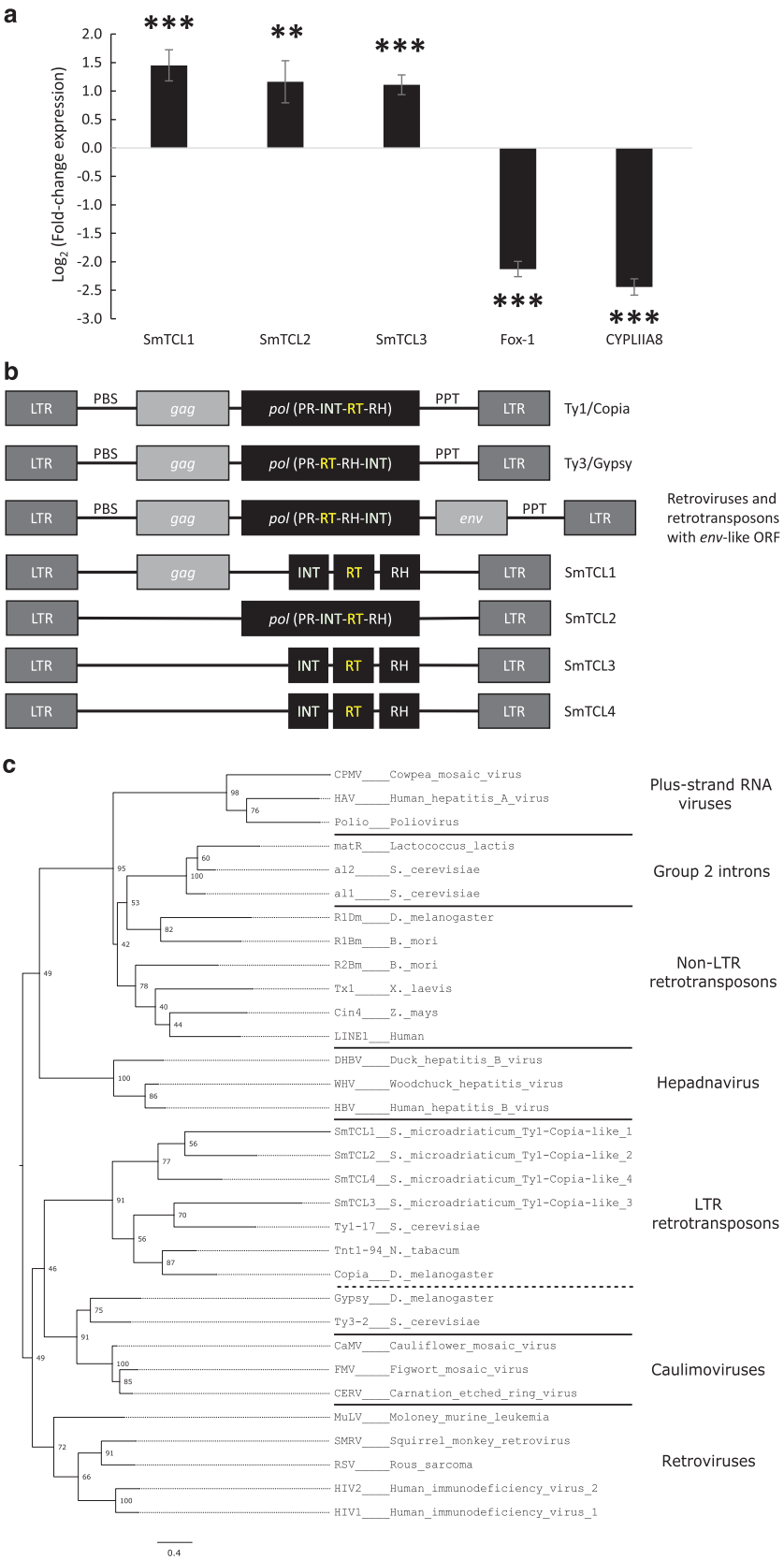
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Ty3/Gypsy
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SmTCL2
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SmTCL3
LTR
INT
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RH
LTR
SmTCL4
c
CPMV____Cowpea_mosaic_virus
HAV_____Human_hepatitis_A_virus
Polio___Poliovirus
Plus-strand RNA viruses
matR____Lactococcus_lactis
a12_____S._cerevisiae
a11_____S._cerevisiae
Group 2 introns
R1Dm____D._melanogaster
R1Bm____B._mori
R2Bm____B._mori
Tx1_____X._laevis
Cin4____Z._mays
LINE1___Human
Non-LTR retrotransposons
DHBV____Duck_hepatitis_B_virus
WHV_____Woodchuck_hepatitis_virus
HBV_____Human_hepatitis_B_virus
Hepadnavirus
SmTCL1__S._microadriaticum_Tyl-Copia-like_1
SmTCL2__S._microadriaticum_Tyl-Copia-like_2
SmTCL4__S._microadriaticum_Tyl-Copia-like_4
SmTCL3__S._microadriaticum_Tyl-Copia-like_3
Ty1-17__S._cerevisiae
Tnt1-94_N._tabacum
Copia___D._melanogaster
LTR retrotransposons
Gypsy___D._melanogaster
Ty3-2___S._cerevisiae
CaMV____Cauliflower_mosaic_virus
FMV_____Figwort_mosaic_virus
CERV____Carnation_etched_ring_virus
Caulimoviruses
MuLV____Moloney_murine_leukemia
SMRV____Squirrel_monkey_retrovirus
RSV_____Rous_sarcoma
HIV2____Human_immunodeficiency_virus_2
HIV1____Human_immunodeficiency_virus_1
Retroviruses
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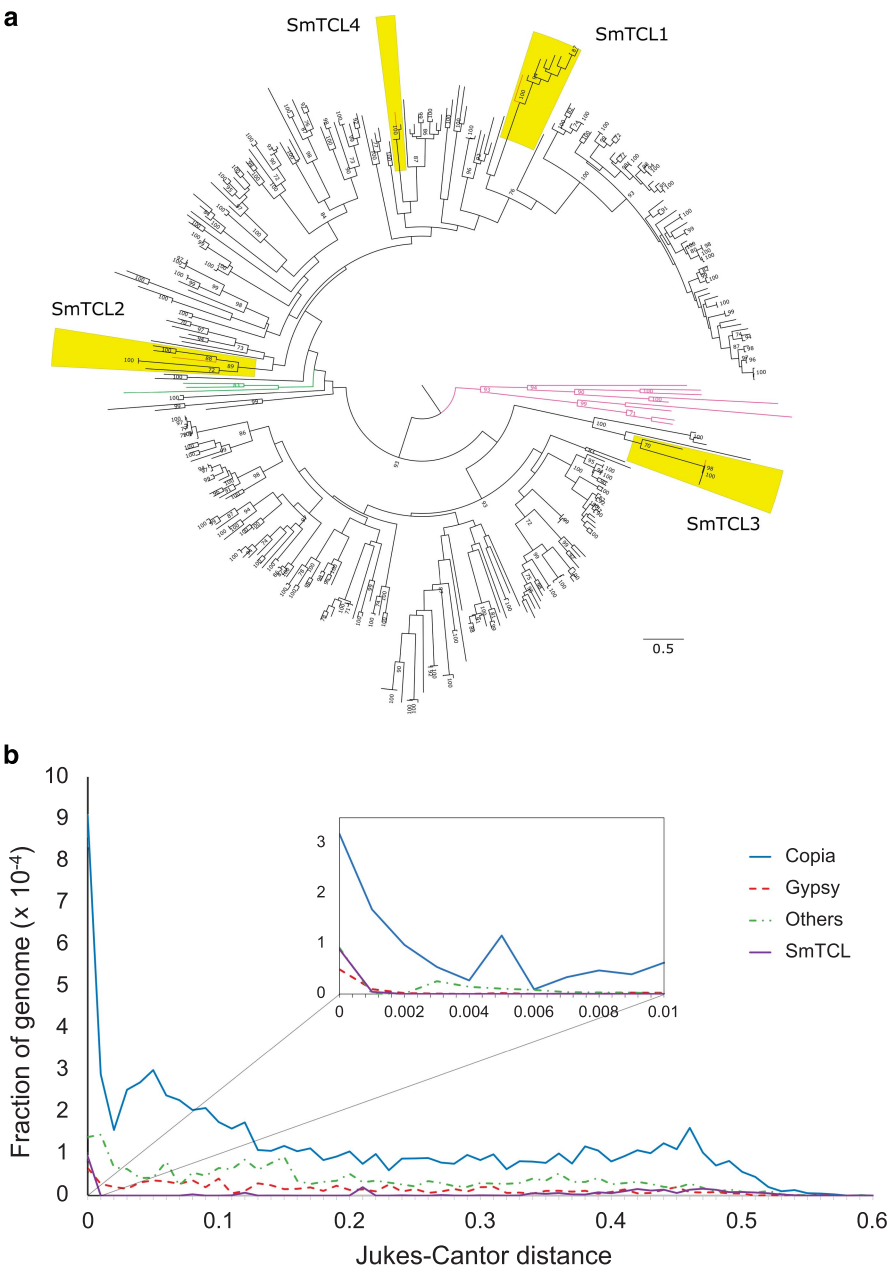

Figure 2 Genome-wide analyses of *copia*-like elements. (**a**) Maximum likelihood phylogeny of 365 reverse transcriptase amino-acid sequences curated *in silico* from the *S. microadriaticum* genome. Green lines indicate known Ty1-*copia*-type sequences, orange lines indicate SmTCL1-4, pink lines indicate retrovirus, caulimovirus and Ty3-*gypsy*-like outgroup sequences as seen in Figure 1c and Supplementary Figure S1, black lines indicate Ty1-*copia*-like sequences. Sequences considered to form the SmTCL subclades are highlighted in yellow. Bootstrap values below 70 were removed from the phylogeny. Scale bar indicates number of substitutions per site. (**b**) Jukes–Cantor distribution of selected retrotransposon families in the *S. microadriaticum* genome. Insert plot shows the distribution of retrotransposons within the 0–0.01 Jukes–Cantor distance fraction, at bin sizes of 0.001. SmTCL (purple line) shows the genome coverage of sequences associated with the heat responsive elements SmTCL1-4. A sequence with 50% of its nucleotide positions different from its consensus sequence would have a Jukes–Cantor distance of 0.824 according to the equation, $d = -\left(\frac{3}{4}\right) \times \ln\left(1 - \left(\frac{4}{3}\right)p\right)$, where p is the proportion of sites with different nucleotides and d is the Jukes–Cantor distance.

Figure 1 Evidence for putative heat stress-activated Ty1-*copia*-like retrotransposons. (**a**) qPCR expression analysis of selected genes under heat stress. SmTCL, *S. microadriaticum* Ty-*copia*-like; Fox-1, RNA-binding protein fox-1 homolog; CYPLIIA8, Cytochrome P450. Error bars show the standard error of the means. **one-tailed *t*-test $P < 0.01$; ***$P < 0.001$. (**b**) Domain arrangements of various LTR retroelements. LTR, long terminal repeats; PBS, primer binding site; gag, group-specific antigen; pol, pol polyprotein; PR, protease; INT, integrase; RT, reverse transcriptase; RH, ribonuclease H; PPT, polypurine tract; env, envelope protein. (**c**) Maximum likelihood phylogeny of 32 reverse transcriptase or RNA-dependent RNA polymerase amino-acid domains of various retrotransposons and viruses. Dotted lines indicate polyphyletic groupings and the scale bar indicates number of substitutions per site. Node support values indicate bootstrap support.

phenotypes via retrotransposon-mediated mutagenesis (Ito *et al*., 2011, 2016).

We have identified four sequences in the *Symbiodinium microadriaticum* genome (Aranda *et al*., 2016) as putative Ty1-*copia* class LTR retrotransposons using previously published transcriptomic data (Liew *et al*., 2017), henceforth referred to as *S. microadriaticum Ty1-Copia-Like* (*SmTCL*) 1, 2, 3 and 4. The first two were identified as being the two most upregulated genes in response to 36 °C temperature stress (Supplementary Table S1) in the assembled transcriptome. In addition, we identified two further heat stress-activated SmTCLs by conducting an independent differential expression analysis on a set of genome-curated sequences that were pre-identified as Ty1-*copia* sequences (Supplementary Table S2).

To confirm that the overexpression of these sequences was indeed a direct consequence of the heat stress treatment, we repeated the RNA extraction experiment twice and validated the differential expression of SmTCL1, 2 and 3 using quantitative reverse transcription polymerase chain reaction (RT-qPCR) (Figure 1a). All three SmTCLs were significantly upregulated in heat-stressed samples. In addition, we also tested the expression of Fox-1 and CYPLIIA8, which were the two most downregulated transcripts in our RNA-Seq data set (Supplementary Table S1).

Analysis of the genetic structure showed that all four *SmTCL*s possess a reverse transcriptase (RT) domain, an integrase domain upstream of said RT domain, a RNase H domain and are flanked by LTRs (Figure 1b). The position of the integrase domain plays a key role in the classification of the two major classes of LTR retrotransposons: Ty1-*copia* and Ty3-*gypsy*. The domain arrangement of Ty3-*gypsy* is very similar to that of retroviruses (Figure 1b), with the integrase domain being downstream of the reverse transcriptase domain. We also built a phylogenetic tree (Figure 1c) using the aligned amino-acid sequences of 28 model reverse transcriptases (Supplementary Figure S1) from a diverse variety of retroelements (Xiong and Eickbush, 1990) in order to classify the *SmTCL*s. We found that all *SmTCL*s clustered together with the Ty1-*copia* family of retrotransposons. To our knowledge, this is the first reported example of heat-activated retrotransposons in *S. microadriaticum* in particular, and dinoflagellates in general.

We determined the prevalence of *SmTCL* RT isoforms in the genome using genome-wide BLAST analysis and identified 343 RT sequences, which were classified as Ty1-*copia*-like sequences based on phylogenetic analysis relative to a retrovirus and Ty3-*gypsy* outgroup (Figure 2a). The *SmTCL*s grouped separately, indicating that these sequences diverged some time ago.

A genome-wide analysis of the Jukes–Cantor distribution of LTR retrotransposons (Chapman *et al*., 2010) in the *Symbiodinium* genome indicates that *copia*-like sequences have had a recent increase in copy numbers, as evidenced by a large number of elements showing almost no sequence divergence (0–0.01 Jukes–Cantor distance) (Figure 2b). In terms of genome coverage, the plurality of *copia* sequences are considered near-identical to their consensus sequences indicating that they have not had the time to acquire nucleotide changes. Interestingly, we found that the heat responsive elements SmTCLs1–4 constituted ~26% of the genome coverage of the lowest divergence fraction (0–0.001 Jukes–Cantor distance), indicating that these elements expanded recently (Supplementary Table S3). For SmTCL3, for instance, we identified five near-identical full-length copies (>99.9% similarity over ~10 kb) as well as a 500 bp copy with the same LTRs (Supplementary Data S1).

In the diatom *Phaeodactylum tricornutum*, expression of the Ty1-*copia*-like retrotransposons *Blackbeard* was found to be induced by nitrate starvation (Maumus *et al*., 2009). Different accessions of *P. tricornutum* were shown to possess different retrotransposition events that were distinguishable at the population level. Like *Symbiodinium*, *P. tricornutum* has not been directly observed to undergo sexual reproduction (Levin *et al*., 2016). Unlike *Phaeodactylum*, *Symbiodinium* has a haploid genome (Santos and Coffroth, 2003) and thus retrotransposition into a functional genomic region would likely have a stronger phenotypic effect. Retrotransposon-mediated stress-activated mutagenesis could thus be a mechanism to generate additional genetic variation under adverse conditions, where the cost of deleterious mutations is outweighed by the possibility of acquiring novel adaptations (Rey *et al*., 2016).

Our results do not provide evidence for new, heat stress-induced retrotransposition events, but they suggest that mutation rates for *S. microadriaticum* could increase substantially due to stress-mediated transcriptional activation of transposons, leading to a greater-than-expected increase in genetic variation under acute heat stress (Chakravarti *et al*., 2017). Further work investigating whether isolated, post-heat-stressed *S. microadriaticum* possess more LTR retrotransposon copy numbers compared to non-stressed control cultures would be the first step to establishing whether these new retrotransposition events are capable of generating new phenotypes from a clonal parental population. This would provide some indication whether it would be feasible to generate phenotypes with increased coral symbiosis resilience to heat stress via heat stress-induced 'experimental evolution' of *S. microadriaticum* strains (van Oppen *et al*., 2017). Additionally, *in natura* analyses comparing LTR retrotransposon copy numbers in *Symbiodinium* populations from sites differing in temperature fluctuations could provide further evidence for the ecological relevance of such a mechanism under natural conditions.

Conflict of Interest

The authors declare no conflict of interest.

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