High expression of the breast cancer susceptibility gene BRCA1 in long-lived termite kings

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ABSTRACT

Aging is associated with the accumulation of DNA damage. High expression of DNA repair genes has been suggested to contribute to prolonged lifespan in several organisms. However, the crucial DNA repair genes contributing to longevity remain unknown. Termite kings have an extraordinary long lifespan compared with that of non-reproductive individuals such as workers despite being derived from the same genome, thus providing a singular model for identifying longevity-related genes. In this study, we demonstrated that termite kings express higher levels of the breast cancer susceptibility gene BRCA1 than other castes. Using RNA sequencing, we identified 21 king-specific genes among 127 newly annotated DNA repair genes in the termite *Reticulitermes speratus*. Using quantitative PCR, we revealed that some of the highly expressed king-specific genes were significantly upregulated in reproductive tissue (testis) compared to their expression in somatic tissue (fat body). Notably, BRCA1 gene expression in the fat body was more than 4-fold higher in kings than in workers. These results suggest that BRCA1 partly contributes to DNA repair in somatic and reproductive tissues in termite kings. These findings provide important insights into the linkage between BRCA1 gene expression and the extraordinary lifespan of termite kings.

INTRODUCTION

Genomic instability and DNA damage are hallmarks of aging and number of aging-linked diseases in multi-cellular organisms [1]. Indeed, the frequency of DNA damage increases with age in humans, rodents, and flies [2,3]. Aging-associated DNA damage is normally repaired by various DNA repair proteins [4]. Thus, these proteins are highly conserved in many organisms, and they play important roles in organismal aging and

longevity [4]. In mammals, longer-lived species such as humans and naked mole rats exhibit higher expression of DNA repair genes than short-lived mice [5]. Moreover, overexpression of several DNA repair genes contributes to the extension of lifespan in the fly *Drosophila melanogaster* [6]. These studies suggest that efficient DNA repair system contributes to extended longevity. However, the crucial DNA repair genes contributing to extraordinary longevity are unclear.

In eusocial insects, such as ants, honeybees, and termites, the lifespan of queens is more than 10-fold longer than that of non-reproductive individuals (workers and soldiers) possessing the same genome, a fact that has attracted significant attention [7–9]. In contradistinction to the queens, reproductive male ants and honeybees have a relatively short lifespan (2–3 months) [10,11]. Conversely, termite kings exhibit extraordinary longevity similarly as the queens [12]. This illustrates that termite kings have an exceptional anti-aging system. A previous study focusing on somatic tissues reported that long-lived queens displayed higher expression of DNA repair genes than short-lived workers in the ant *Lasius niger* [13]. These findings suggest that highly efficient DNA repair mechanisms can also contribute to the extraordinary longevity of termite kings.

The Japanese subterranean termite *Reticulitermes speratus* is one of the most studied termites due to its reproductive system [14]. A novel parthenogenetic system known as asexual queen succession (AQS) was revealed in *R. speratus* [15]. AQS allows queens to extend their reproductive lifespan because a founding queen (primary queen; PQ) is genetically immortal until a colony dies due to the production of a large number of nest queens (secondary queens; SQs) through parthenogenesis. Conversely, a founding king (primary king; PK) should continue to live even if queens are replaced because mother–son inbreeding depression can occur when the PK dies, reducing the genetic contribution to offspring [14,16,17]. Indeed, a previous study has revealed that this king replacement does not often

occur in wild colonies of *R. speratus* [18]. It is thus presumed that PKs in *R. speratus* have been exposed to selection for inbreeding avoidance. As a previous study reported that *Reticulitermes* termite queens surprisingly live more than 11 years [19], the PKs of *R. speratus* may have the potential to live for several decades. Therefore, *R. speratus* provides an ideal model for identifying the critical DNA repair genes contributing to extended longevity.

In this study, we performed transcriptome analysis of DNA repair genes using RNA sequencing (RNA-seq) data obtained from all castes of *R. speratus* (both females and males workers, soldiers, alates, young PQs and PKs, and mature SQs and PKs) [20]. We additionally investigated age-dependent expression changes in male reproductives (male alates and young and mature PKs). Furthermore, we investigated where DNA repair genes were overexpressed in the termite body using quantitative PCR (qPCR) analysis. This study uncovered novel evidence that DNA repair functions as an anti-aging mechanism in termite kings.

RESULTS

Digital transcriptome analyses of DNA repair genes in *R. speratus*

Aging-associated DNA damage including abnormal bases, abasic sites, 8-oxoguanine, single-strand breaks (SSBs), bulky adducts, pyrimidine dimers, double-strand breaks (DSBs), interstrand cross-links, and genomic mismatches are generally repaired by various
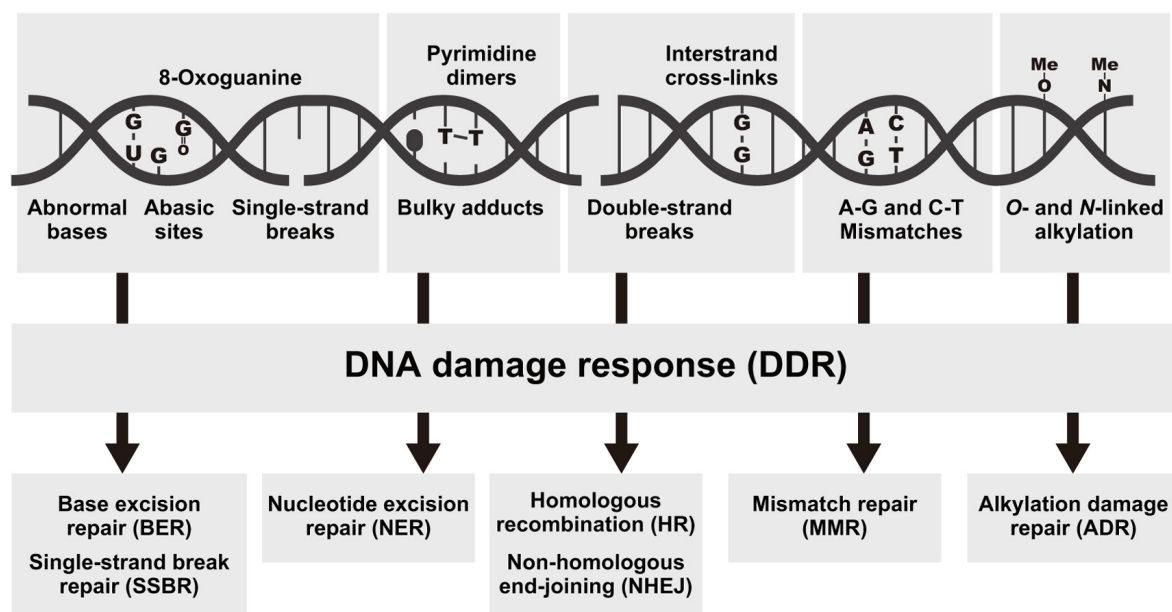

Figure 1. Types of DNA damage and the associated repair pathways. Examples of DNA lesions (top), activation of the DNA damage response (DDR, middle), and the most relevant DNA repair pathways responsible for the removal of the lesions (bottom).

pathways such as base excision repair (BER), SSB repair (SSBR), nucleotide excision repair (NER), homologous recombination (HR), non-homologous end-joining (NHEJ), mismatch repair (MMR), and alkylation damage repair (ADR) [4,21–26] (Fig. 1). Using RNA-seq, we identified 127 putative DNA re-

pair genes including 50 DNA damage response (DDR) genes, 26 HR genes, 16 NER genes, 11 NHEJ genes, 9 ADR genes, 7 MMR genes, 4 genes related to various DNA repair-related pathways, 3 BER genes, and 1 SSBR gene in *R. speratus* (Tables S1 and S2).
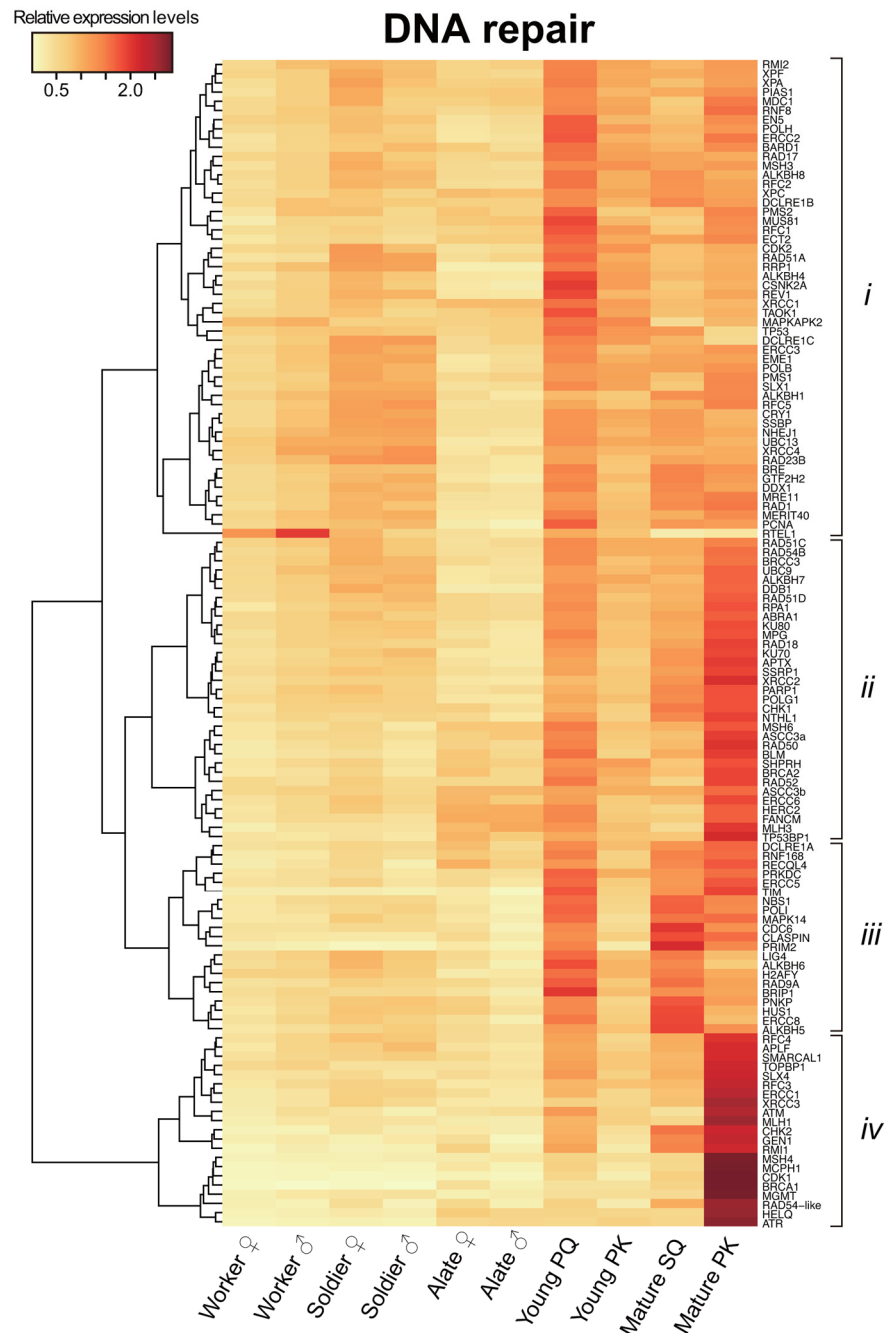

Figure 2. Differential transcriptome analysis of DNA repair genes among castes. The heatmap shows the differential expression of 127 DNA repair transcripts among castes in *Reticulitermes speratus*. Workers and soldiers are non-reproductive individuals. Alates, young primary queens (PQs) and primary kings (PKs), and mature secondary queens (SQs) and PKs are reproductives. After nuptial flight, a pair of female and male alates establishes a new colony and starts to produce offspring sexually as a PQ and PK, respectively. The relative expression level is indicated by the mean normalized count per million, ranging from white to red. The tree at the left corresponds to the hierarchical clustering of cluster-averaged expression (clusters *i–iv*).

Digital transcriptome analysis indicated that the expression of 97 genes differs among castes (FDR < 0.05; Fig. 2 and Table S3). We then identified four clusters of genes with significantly varied expression among castes (clusters *i–iv*). The clusters had tendencies for high gene expression in young PQs (cluster *i*); young PQs and mature PKs (cluster *ii*); young PQs, SQs and mature PKs (cluster *iii*); or mature PKs (cluster *iv*). Consequently, we identified 21 mature PK-specific genes including nine HR repair genes, six DDR genes, two NHEJ repair genes, two MMR genes, one NER genes, and one ADR gene in cluster *iv* (Table 1). Moreover, principal component analysis (PCA) largely confirmed the results of clustering (Fig. S1 and Table S4). Thus, RNA-seq analysis revealed that mature PKs had superior DDR and HR repair pathways relative to the other castes (Fig. 3A and 3B). MMR and ADR signals are also activated in PKs (Fig. 3C). Moreover, we revealed that the expression of 93 genes (including 40 DDR genes, 19 HR genes, 9 NER genes, 7 NHEJ genes, 6 ADR genes, 5 MMR genes, 4 genes related to various DNA repair-related pathways, and 3 BER genes) increases significantly with age in male reproductives (Table S5). These 93 genes included the aforementioned 21 mature PK-specific genes.

Caste-specific expression analysis of DNA repair genes

We performed whole-body qPCR analysis to investigate whether termite kings have higher DNA repair gene expression than other castes. We focused on BRCA1, MCPH1, MHL1, and XRCC3 among mature PK-specific genes because these genes had higher expression in cluster *iv* and they play important roles in genome maintenance. Whole-body qPCR revealed that mature PKs and SQs have higher expression of *RsBRCA1*, *RsMCPH1*, and *RsMLH1* than workers and soldiers (n = 6, *P* < 0.05; Figs. 4A–C). *RsXRCC3* was highly expressed only in PKs (n = 6, *P* < 0.05; Fig. 4D).

Table 1. King-specific gene list.

Gene symbol	Full gene name	Signal
RFC3	Replication factor C subunit 3	DDR
RFC4	Replication factor C subunit 4	DDR
TOPBP1	DNA topoisomerase 2-binding protein 1	DDR
ATR	Serine/threonine-protein kinase ATR	DDR
ATM	Serine-protein kinase ATM	DDR
CHK2	Serine/threonine-protein kinase Chk2	DDR
ERCC1	DNA excision repair protein ERCC-1	NER
BRCA1	Breast cancer type 1 susceptibility protein	HR
MCPH1	Microcephalin	HR
XRCC3	X-ray repair cross-complementing protein 3	HR
CDK1	Cyclin-dependent kinase 1	HR
HELQ	Helicase POLQ-like	HR
RMI1	RecQ-mediated genome instability protein 1	HR
GEN1	Flap endonuclease GEN	HR
SLX4	Structure specific endonuclease subunit SLX4	HR
RAD54-like	DNA repair and recombination protein RAD54-like	HR
APLF	Aprataxin and PNK-like factor	NHEJ
SMARCAL1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1	NHEJ
MLH1	DNA mismatch repair protein Mlh1	MMR
MSH4	MutS protein homolog 4	MMR
MGMT	Methylated-DNA—protein-cysteine methyltransferase	ADR

DDR, DNA damage response; NER, nucleotide excision repair; HR, homologous recombination; NHEJ, non-homologous end-joining; MMR, mismatch repair; ADR, alkylation damage repair. The genes written in bold were used for quantitative PCR analysis in this study.
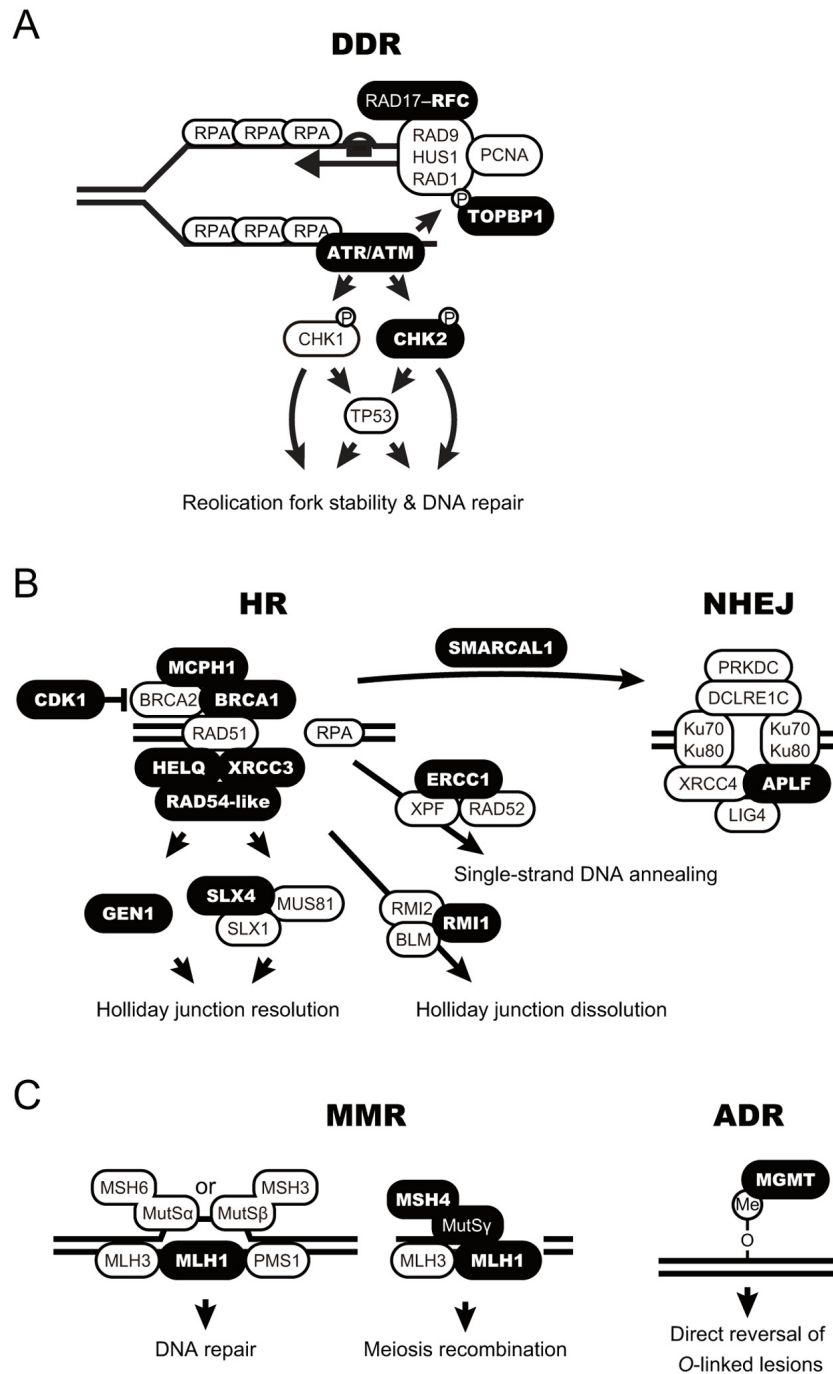
Figure 3. Schematic model for accelerated DNA repair pathways in termite kings. (**A**-**C**) DNA damage response (DDR; **A**), homologous recombination and non-homologous end-joining (HR and NHEJ, respectively; **B**), and mismatch repair and alkylation damage repair (MMR and ADR, respectively; **C**) are shown as schematic models. The proteins encoded by the king-specific genes (cluster *iv* in Figure 2) are highlighted in bold.

Tissue-specific expression analysis in termite workers and reproductives

To determine whether these repair genes are expressed in the termite body, we performed tissue-specific qPCR analysis in workers and mature reproductives. The testis of PKs displayed the highest *RsBRCA1* expression among all termite tissues (n = 6, *P* < 0.05; Fig. 5A). In addition, the fat body of PKs displayed higher *RsBRCA1* expression than that of workers and SQs (n =

6, *P* < 0.05; Fig. 5A). *RsMCPH1* expression was higher in the ovaries and testes than in the fat body except in all castes excluding PKs (n = 6, *P* < 0.05; Fig. 5B). Meanwhile, *RsMLH1* and *RsXRCC3* expression was higher in the ovaries and testes than in the fat body (n = 6, *P* < 0.05; Figs. 5C and 5D). Furthermore, we assessed the expression levels of *RsCDK1* and *RsMGMT*, which are also considered as mature PK-specific genes, and these tended to be higher in the ovaries and testes than in the fat body, but there were no statistically significant differences (n = 6; Figs. 5E and 5F).

DISCUSSION

Our analysis demonstrated that mature PKs exhibit higher expression of the BRCA1 gene than other castes in *R. speratus*. BRCA1 is one of the most studied DNA repair genes, particularly in cancer research. Mutation of the gene is linked to familial breast and ovarian cancers [27]. To date, BRCA1 has been implicated in various biological processes, including microRNA biosynthesis [28], antioxidant signaling [29], and homologous DNA repair [30]. However, the role of BRCA1 in organismal
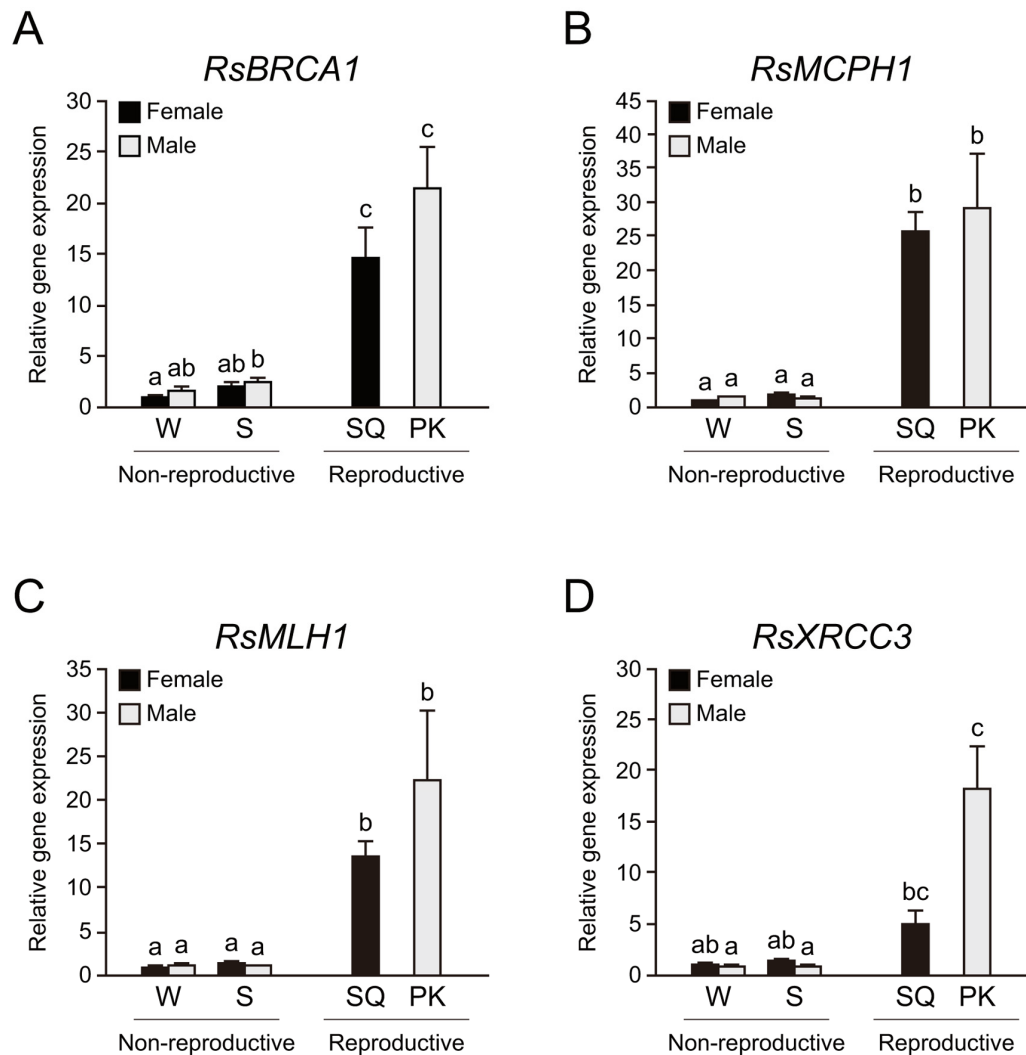

Figure 4. Higher expression of DNA repair genes in termite reproductives. (**A**) There were significant differences in *RsBRCA1* expression among castes of *Reticulitermes speratus* (n = 6; *P* < 0.001). *RsBRCA1* expression is higher in reproductives than in non-reproductives (n = 6; *P* < 0.05). (**B**) There were significant differences in *RsMCPH1* expression among castes of *R. speratus* (n = 6; *P* < 0.001). *RsMCPH1* expression is higher in reproductives than in non-reproductives (n = 6; *P* < 0.05). (**C**) There were significant differences in *RsMLH1* expression among castes of *R. speratus* (n = 6; *P* < 0.001). *RsMLH1* expression is higher in reproductives than in non-reproductives (n = 6; *P* < 0.05). (**D**) There were significant differences in *RsXRCC3* expression among castes of *R. speratus* (n = 6; *P* < 0.001). *RsXRCC3* expression is higher in PKs than in non-reproductives (n = 6; *P* < 0.05). Black and gray bars indicate female and male individuals, respectively. Error bars represent the standard error of the mean. Different letters (a–c) over the bars denote significant differences at *P* < 0.05. W, workers; S, soldiers; SQ, mature secondary queens; PK, mature primary kings.
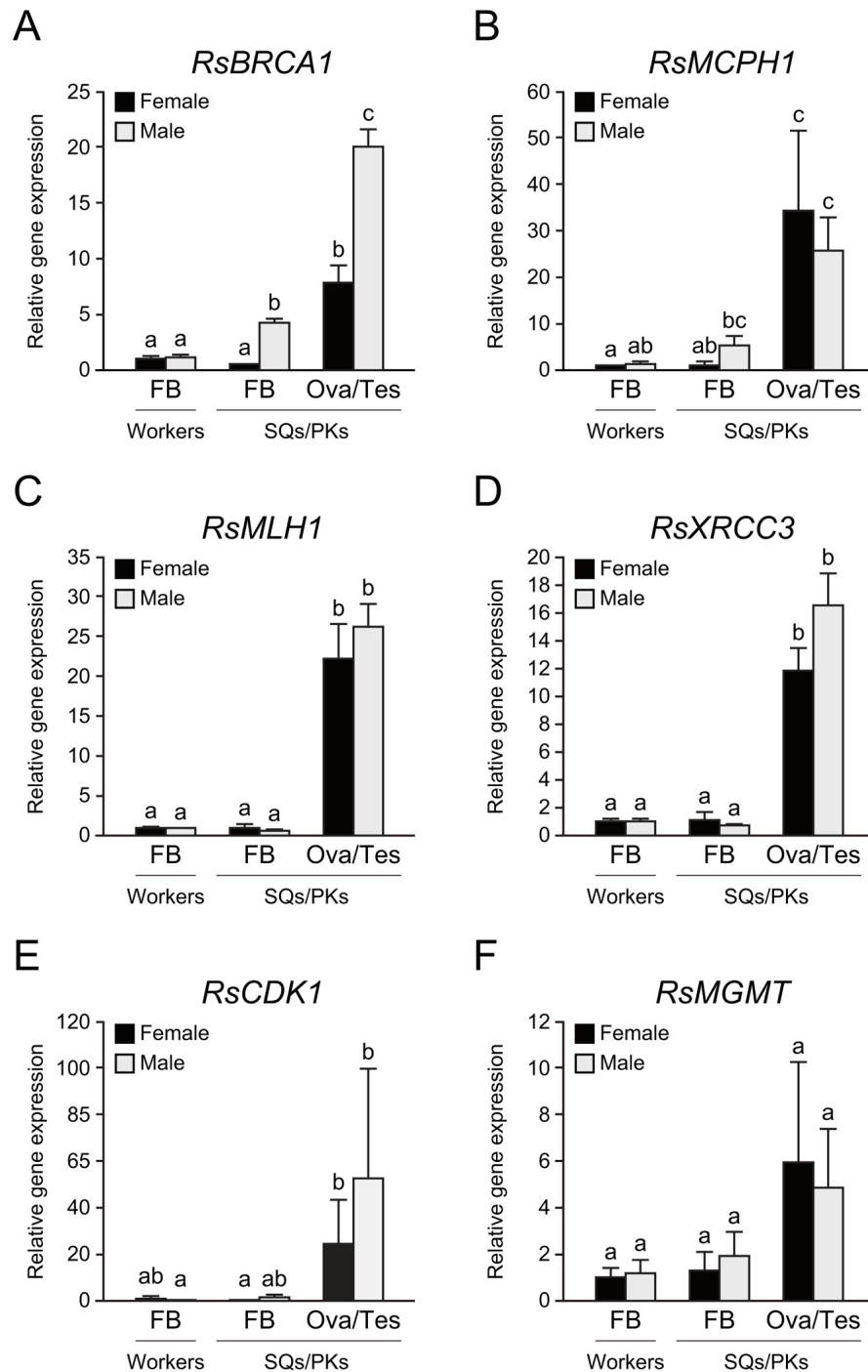
Figure 5. Tissue-specific expression of several DNA repair genes in termite workers and reproductives. (**A**) There were significant differences in *RsBRCA1* expression between these tissues (n = 6; *P* < 0.001). *RsBRCA1* expression is higher in testes than in ovaries and the fat body (n = 6; *P* < 0.05). *RsBRCA1* expression is higher in the fat body of PKs than in that of workers and SQs (n = 6; *P* < 0.05). (**B**) There were significant differences in *RsMCPH1* expression between these tissues (n = 6; *P* < 0.001). *RsMCPH1* expression is higher in ovaries and testes than in the fat body of workers and SQs (n = 6; *P* < 0.05). (**C**) There were significant differences in *RsMLH1* expression between these tissues (n = 6; *P* < 0.001). *RsMLH1* expression is higher in the ovaries and testes than in the fat body of workers and reproductives (n = 6; *P* < 0.05). (**D**) There were significant differences in *RsXRCC3* expression between these tissues (n = 6; *P* < 0.001). *RsXRCC3* expression is higher in the ovaries and testes than in the fat body of workers and reproductives (n = 6; *P* < 0.05). (**E**) There were significant differences in *RsCDK1* expression between these tissues (n = 6; *P* < 0.001). *RsCDK1* expression was higher in the ovaries and testes than in the fat body of workers and reproductives (n = 6; *P* < 0.05). (**F**) Although there were significant differences in *RsMGMT* expression between these tissues (n = 6; *P* < 0.05), post-hoc tests revealed no significant differences (n = 6). Black and gray bars indicate females and males, respectively. Error bars represent the standard error of the mean. Different letters (a–c) over the bars denote significant differences at *P* < 0.05. FB, fat body; Ova, ovary; Tes, testis.

aging and longevity has remained mostly unclear. The present study suggests that BRCA1 plays an important role in the extraordinary longevity of termite kings. Our findings suggest that BRCA1 can partly contribute to efficient DNA repair in both somatic and reproductive tissues in termite kings.

BRCA1 is known to have a role of RAD51-mediated HR repair [30]. A previous study reported that the accumulation of RAD51 onto resected DSBs is the cause of HR defects in older flies [3]. Thus, our results indicated that BRCA1 may be a key upstream regulator of the age-related accumulation of RAD51 and the subsequent HR defects. Consistent with this finding, BRCA1 gene overexpression can reduce age-related DSB accumulation and increase cell survival in mouse oocytes [31]. This data provides support for the assertion that BRCA1-regulated HR strongly influences DNA repair efficiency in termite kings with age.

BRCA1, MCPH1, XRCC3, and MLH1 are known to have roles in meiotic progression and the maintenance of genomic stability [32–35]. The germline genome is vulnerable to the accumulation of deleterious mutations during meiotic DNA replication. If those mutations are not eliminated in reproductive cells, errors could be passed to offspring, which is linked to an increased risk of diseases in future generations [36]. Therefore, our findings indicate that strong evolutionary pressures potentially led to the high expression of DNA repair genes in reproductive tissues, especially in the long-lived organisms having the long reproductive periods. Indeed, long-lived humans display lower germline mutation rates than short-lived mice due to high DNA repair gene expression [37]. Additionally, DNA damage levels were lower in long-lived termite queens than in short-lived workers [38]. It is necessary to examine whether mature PKs and SQs have lower levels of DNA damage in reproductive tissues than short-lived species in future research.

The fat body plays major roles in metabolic and reproductive functions in insects [39]. Increased metabolism and reproduction are considered to generate oxidative stress [40–42], which is known to be a cause of DNA damage [43]. The cost of egg production is generally higher than the cost of sperm production [44], suggesting that the fat body of termite queens with long-term reproductive activity may be exposed to strong oxidative stress in comparison to that of the kings. Nevertheless, we found that the fat body of termite kings expressed higher levels of *RsBRCA1* than that of the queens in the present study. Furthermore, we demonstrated that the fat body of the kings does not show high expression levels of *RsCDK1*, which is critical for M-phase entry in mitosis [45]. These

findings indicate the possibility that an increased BRCA1 expression is associated with greater longevity and not with cellular stress caused by increased metabolism, reproduction, and mitosis in the fat body of termite kings.

Previous studies described that the long longevity and survival of termites are associated with efficient anti-oxidant systems [38,46,47]. Thus, although the antioxidant system of termite kings remains unclear, it is estimated that an efficient antioxidant system can prevent the accumulation of DNA damage and con-tribute to the greater longevity of the kings. Interestingly, a recent study has identified that long-lived reproductives have strong defense systems against transposons as a potential source of DNA damage in comparison to short-lived major workers in the termite *Macrotermes bellicosus* [48]. In addition, long-lived ant queens reportedly display a higher expression of DNA repair genes than short-lived workers [13]. These findings support the hypothesis that the extraordinary longevity of organisms is associated with increased biomolecule homeostasis. However, it is necessary to carefully assess how the reproductives achieve longer lifespans than the non-reproductive individuals in eusocial insects, which has been argued in some studies [49–51]. Therefore, further studies are required to evaluate the biological function of BRCA1 in the lon-gevity of termites using genetic tools, such as CRISPR-Cas9, RNA interference, and transgenic systems. Overall, our study provides new insights into the maintenance mechanisms of biomolecule homeostasis and their links to extraordinary longevity.

MATERIALS AND METHODS

Sample collection

Animal ethics committee approval was not required for this study, which used insects. For RNA-seq analysis, alates were extracted from three colonies in secondary forests in Kyoto, Japan, from April to May 2013. One male and one female alate were randomly selected from each colony, and five pairs were created for each colony and kept at 25°C under darkness. They were extracted from each incipient colony after 6 months, and they were regarded as young PQs and PKs. Workers, soldiers, and mature SQs and PKs were collected from four colonies in secondary forests in Kyoto and Shiga, Japan, from July to October 2013. For qPCR analysis, 12 *R. speratus* colonies (workers, soldiers, and mature SQs and PKs) were collected from secondary forests in Yamaguchi and Shiga, Japan, from May to June in 2016 and 2017. Because of the rare appearance of mature PQs in nature [15], we used SQs as mature queens in this study. Whole bodies or separated tissues (reproduc-

tive tissues and fat body) of the termites were temporarily preserved at −80°C. Female and male workers and soldiers were separated on the basis of the shape of the caudal sternite using a stereoscope. *R. speratus* is not an endangered or protected species; thus, no specific permits were required for the described field studies, and no specific permissions were required for the locations for termite sampling given that they are public lands.

De novo transcriptome assembly

We analyzed a transcriptomic database of both male and female of workers, soldiers, and alates, young PQs and PKs, and mature SQs and PKs of *R. speratus*, which was constructed via RNA-seq in our previous study [20], to compare the expression levels of DNA repair-related genes among castes. Total RNA was extracted from the whole body of each individual of each reproductive caste (alates, young PQs and PKs, and mature SQs and PKs) using an RNeasy mini kit (Qiagen) following the standardized instructions from the manufacturer. For workers and soldiers, 10 individuals of each sex were pooled to extract a sufficient amount of RNA, and we performed RNA-seq analysis on a total of 60 samples. In accordance with a previously described procedure [52] for first-strand cDNA synthesis, RNA-seq was performed using the Illumina HiSeq 2000 at the Okinawa Institute of Science and Technology Graduate University. After trimming the raw sequencing reads, the remaining reads from all samples were assembled *de novo* using Trinity version trinityrnaseq_r2012–04–27 [53,54], which generates transcriptomic assemblies from short read sequences using the de Bruijn graph algorithm. In a previous study [20], further details of the methods as well as a summary statistics of assembly are provided. Sequence data were deposited in the DNA Data Bank of Japan under the BioProject PRJDB3531, which contains links and access to sample data through the BioSample SAMD00026264–SAMD00026323 and the Sequence Read Archive DRR030795–DRR030854.

Annotation of DNA repair-related genes

Targets of DNA repair-related genes were selected on the basis of previously reported genes in animals [4]. The peptide sequences of DNA repair-related proteins of *Z. nevadensis*, *Bombyx mori*, and *D. melanogaster* were obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and used as BLAST queries for our peptide database with an e-value cutoff of 1E−60 (Table S1). Analyses of protein families, domains, and motifs were performed using InterProScan in Blast2GO software v5.1.13 [55] (Table S2). Furthermore, to confirm the

identity of the BRCA1 gene *RsBRCA1*, we performed multiple amino acid sequence alignments with CLUSTALW and conducted phylogenetic analyses using the molecular evolutionary genetics analysis software MEGA7 [56] (Fig. S2). Gene evolutionary history was inferred using the maximum likelihood method based on the JTT matrix-based model [57], which is the best model based on the Bayesian information criterion. BRCA1 homologs of mammals (*Homo sapiens* and *Mus musculus*), a worm (*Caenorhabditis elegans*), ants (*Harpegnathos saltator*, *Ooceraea biroi*, *Pogonomyrmex barbatus*, and *Camponotus floridanus*), bees (*Apis florea* and *Dufourea novaeangliae*), wasps (*Copidosoma floridanum* and *Nasonia vitripennis*), sawflies (*Neodiprion lecontei* and *Athalia rosae*), termites (*R. speratus* and *Z. nevadensis*), beetles (*Aethina tumida* and *Dendroctonus ponderosae*), and butterflies (*Papilio machaon*, *B. mori*, *Spodoptera litura*, and *Helicoverpa armigera*) were analyzed.

Abundance estimation and analyses of differential expression

The expression levels of DNA repair genes were estimated using RSEM v1.2.8 [58] separately for the filtered reads from each sample. Raw read counts generated by RSEM were normalized using the trimmed mean of M-value normalization method [59]. Their read counts were then used for differential expression analyses among castes using the "edgeR" package v3.4.2 [60] in R software v3.2.2. Heatmaps and hierarchical clustering (Euclidean distance; Ward's linkage) were generated using "heatmap.2" in the "gplots" package, and PCA was performed using the "prcomp" function in R software v3.2.2.

qPCR

We designed primer pairs for each of the DNA repair genes using Primer3 v1.1.4 [61] (Table S6). Using an RNeasy mini kit, total RNA was extracted individually from the whole bodies and separated tissues of workers and soldiers (three pooled individuals) and from the whole bodies and separated tissues of mature SQs and PKs. cDNA was synthesized from RNA using a PrimeScript™ RT reagent kit (Takara) and preserved at −20°C. qPCR was performed using an Applied Biosystems® StepOne™ system (Thermo) with *Power SYBR™* Green PCR master mix (Thermo). All procedures were performed in accordance with each manufacturer's protocol. Nicotinamide adenine dinucleotide dehydrogenase subunit 5 (ND5) was selected as the reference gene. Relative expression levels were calculated using a typical ΔΔCt method. We performed six colony replicates for this experiment.

Statistical analysis

R software v3.2.2 was used for statistical analyses in qPCR experiments. Normality and homogeneity assumptions for these qPCR data were evaluated by the Shapiro–Wilk test and Bartlett's test, respectively ($P < 0.05$). We used the Kruskal–Wallis test followed by the Mann–Whitney U test with Holm's correction for multiple comparisons. All data in graphs are presented as the mean ± standard error of the mean, and all P values calculated are provided here. The different letters (a–c) over the bars denote significant differences at $P < 0.05$.

AUTHOR CONTRIBUTIONS

E.T. and Y.I. conceived and designed the study. E.T., T.N., K.K., and K.M. collected the biological material. Y.M. and K.K. performed RNA-seq analysis and E.T. annotated DNA repair-related genes. E.T. performed the qPCR analyses. E.T. drafted the manuscript with contributions from Y.M., T.N., K.K., K.M., and Y.I.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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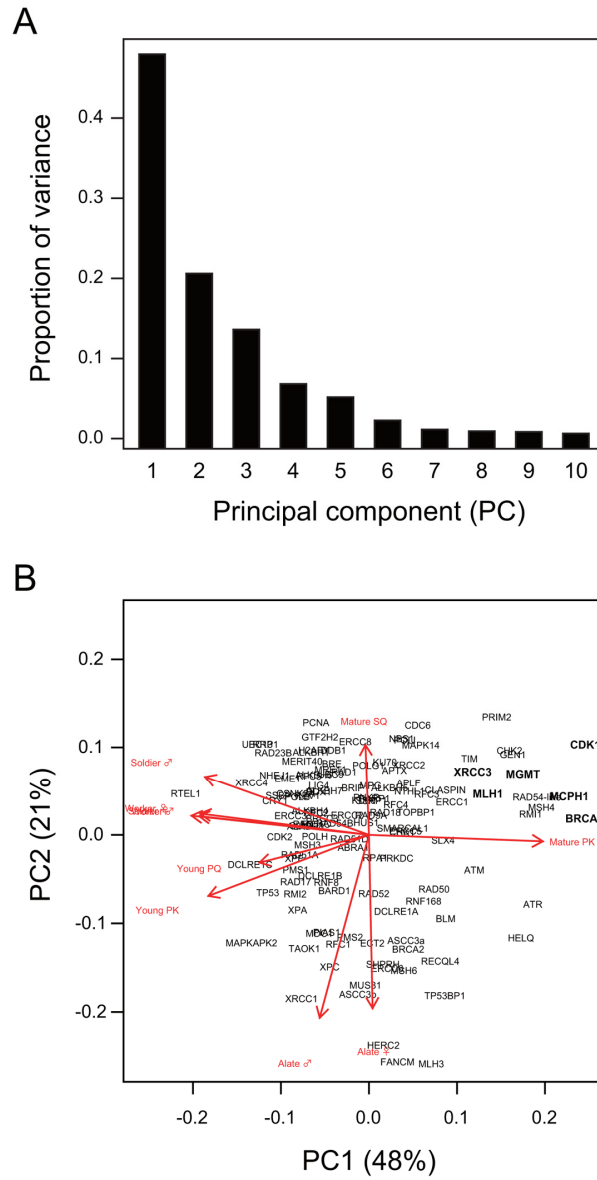
Figure S1. Principal component analysis (PCA) of DNA repair-related gene expression among castes.
(**A**) Scree plot illustrating the proportion of variance accounted for by each PC. (**B**) Scores and loading biplots of DNA repair-related genes and termite castes (red arrows) along PCs are shown. PC1 indicates the mature PK-specific genes (PC factor loadings are provided in Table S4). The genes presented in bold were used for qPCR analysis.
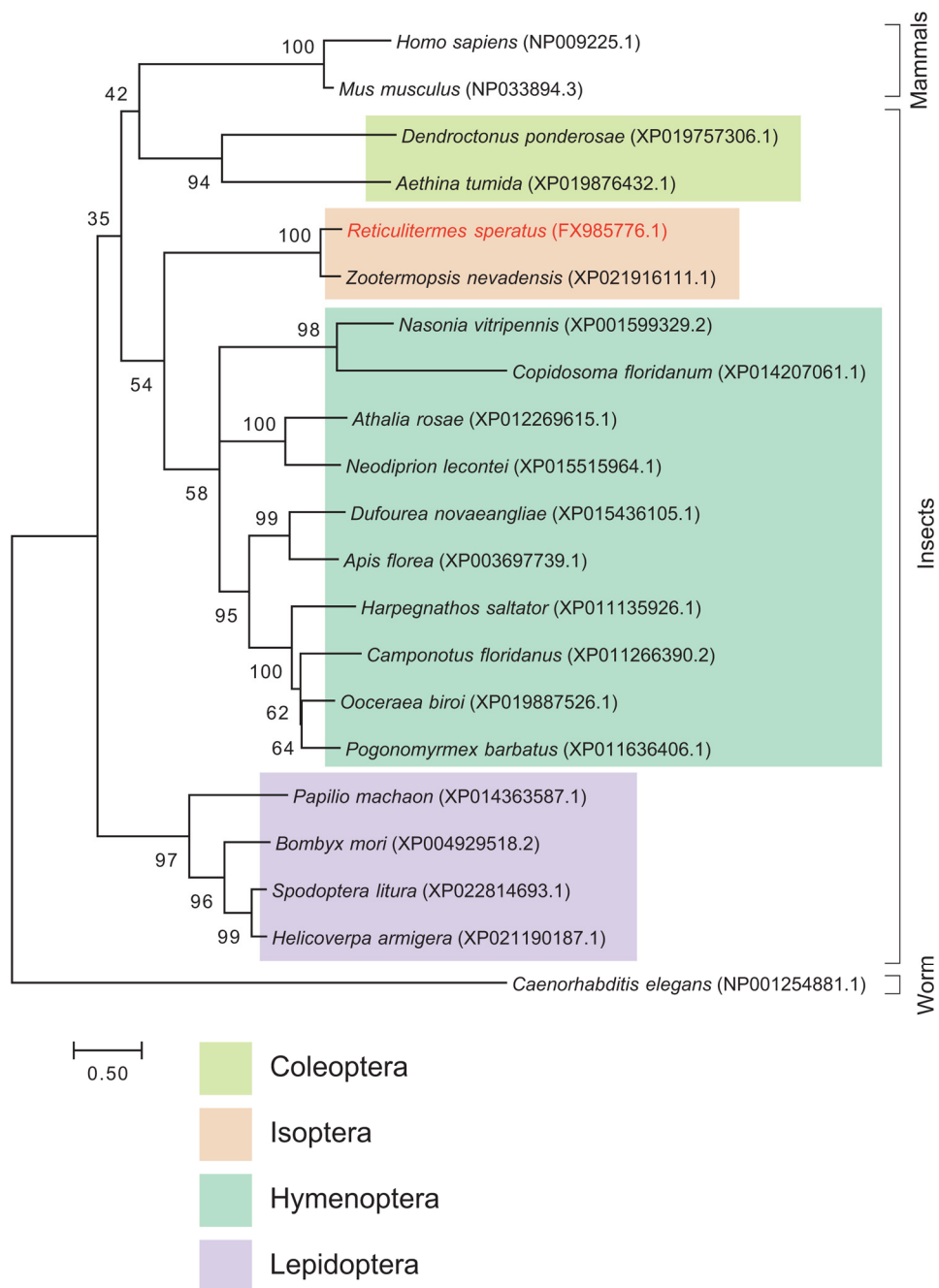
Figure S2. Maximum likelihood molecular phylogenetic tree of BRCA1 homolog sequences. The evolutionary history of the BRCA1 homolog was inferred using the maximum likelihood method based on the JTT matrix-based model. The tree with the highest log likelihood (−9518.81) is shown. The percentage of trees in which the associated taxa clustered together is shown above the branches. Initial tree(s) for the heuristic search were automatically obtained by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model and then selecting the topology with a superior log likelihood value. A discrete gamma distribution was used to model the evolutionary rate differences among the sites (5 categories [+G, parameter = 1.8985]). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 1.34% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 21 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 231.

SUPPLEMENTARY TABLES

Please browse Full Text version to see the data of Supplementary Tables:

Table S1. Target gene information.
Table S2. Predicted protein families, domains, and motifs.
Table S3. Statistical analysis of differential expression among the termite castes.
Table S4. Factor loadings for each principal component.
Table S5. Statistical analysis of age-dependent expression changes in termite kings.
Table S6. Primer sequences.