Gateway to genetic exchange? DNA double-strand breaks in the bdelloid rotifer Adineta vaga submitted to desiccation.

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SEX UNCOVERED SPECIAL ISSUE; Gateway to genetic exchange? DNA double-strand breaks in the bdelloid rotifer *Adineta vaga* submitted to desiccation.

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Running title: genome instability of desiccated bdelloids

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Abstract

The bdelloid rotifer lineage *Adineta vaga* inhabits temporary habitats subjected to frequent episodes of drought. The recently published draft sequence of the genome of *A. vaga* revealed a peculiar genomic structure incompatible with meiosis and suggesting that DNA damage induced by desiccation may have reshaped the genomic structure of these organisms. However, the causative link between DNA damage and desiccation has never been proven to date in rotifers. To test for the hypothesis that desiccation induces DNA double-strand breaks (DSBs), we developed a protocol allowing a high survival rate of desiccated *A. vaga*. Using pulsed-field gel electrophoresis to monitor genomic integrity, we followed the occurrence of DSBs in dried bdelloids and observed an accumulation of these breaks with time spent in dehydrated state. These DSBs are gradually repaired upon rehydration. Even when the genome was entirely shattered into small DNA fragments by proton radiation, *A. vaga* individuals were able to efficiently recover from desiccation and repair a large amount of DSBs. Interestingly, when investigating the influence of UV-A and UV-B exposure on the genomic integrity of desiccated bdelloids we observed that these natural radiations also caused important DNA DSBs, suggesting that the genome is not protected during the desiccated stage but that the repair mechanisms are extremely efficient in these intriguing organisms.

Keywords: DNA damage; asexual reproduction; PFGE; UV radiation; DNA repair; anhydrobiosis, horizontal gene transfer.

Introduction

First described by van Leeuwenhoek in 1702, bdelloid rotifers are microscopic invertebrates that inhabit all types of freshwater environments, with a preference for temporary habitats that dry out frequently. Since their discovery, no males, vestigial male structures or hermaphrodites have ever been observed. Females exhibit a clonal mode of reproduction: they produce eggs through mitotic divisions without apparent chromosome pairing nor reduction in chromosome number (Hsu 1956a, 1956b). These observations brought the controversial hypothesis that bdelloids have been reproducing purely asexually for more than 40 million years (reviewed in Danchin et al. 2011). Recently, the draft sequence of the genome of the bdelloid rotifer *Adineta vaga* yielded positive evidence of an ancient ameiotic evolution: the genome of this species exhibits a peculiar structure in which allelic regions are massively rearranged and sometimes found on the same chromosome in a palindromic fashion. No homologous chromosomes are therefore present in this genome, ruling out the ability of *A. vaga* to perform conventional meiosis (Flot et al., 2013). These various lines of evidence converge on suggesting that bdelloids have been able to survive and diversify for millions of years without sex, yielding more than 400 morphologically defined species (Segers 2007), which makes them of particular interest to evolutionary biologists (Mark Welch and Meselson 2000).

Besides their notorious ancient asexuality, bdelloids are also known for their extreme tolerance to desiccation. At any stage of their life cycle, they can enter a metabolically quiescent state of anhydrobiosis for a prolonged period of time (Ricci et al., 2007). This characteristic enables bdelloid rotifers to colonize limno-terrestial habitats such as mosses and lichens. Thriving in such ephemeral habitat is considered unusual because most animals and plants die instantly if they experience complete desiccation. So far, only species belonging to three animal phyla have been shown to be able to survive and reproduce in ephemeral

habitats: bdelloid rotifers, tardigrades and nematodes. Animals belonging to these clades are therefore model organisms to unravel the mechanisms of desiccation tolerance within the metazoans (Alpert, 2006). In addition, the asexual nature of bdelloid rotifers raises interesting questions regarding a possible interplay between genetic exchange and desiccation (Flot et al. 2013).

The ability to withstand desiccation is a complex phenomenon that takes place at every level of the anatomical and cellular organization. When bdelloids enter desiccation, their bodies contract into a "tun" shape: the body becomes flattened dorso-ventrally and reduced in size, the gut and vitellaria are packed ventrally while the trochi and foot are retracted inside the body (Marotta et al., 2010). At cellular level, survival to desiccation requires adaptations that maintain the function of macromolecules (*e.g.* proteins and DNA) and membranes despite dehydration. Such result can be achieved through two non-exclusive ways: either by preserving the integrity of these molecules, or by repairing them if they have been damaged during the drying process. Sugars and antioxidants are key components that help maintaining the integrity of proteins and membranes in all desiccated beings (Tunnacliffe et al., 2005; França et al 2007). However, several studies have shown that genomic integrity is actually not systematically preserved following desiccation. For instance, prolonged desiccation induces DNA double strand breaks (DSBs) in bacteria such as *Deinococcus radiodurans* (Mattimore & Battista 1996*)* as well as in tardigrades (Neumann et al. 2009) and chironomid larvae (Gusev et al., 2010), which does not impede them to survive anhydrobiosis. A counterexample is cyanobacteria, in which desiccation causes only limited damage to their DNA (Shirkey et al., 2003, Billi 2009). In addition to desiccation resistance, *D. radiodurans* also exhibits an extreme radiation resistance (Mattimore & Battista, 1996). These two observations raised the hypothesis that the ability of *D. radiodurans* to survive both extreme conditions was not coincidental, but linked to very efficient DSBs repair pathway(s)

(Zahradka et al., 2006). Interestingly, hydrated adults of the bdelloid rotifer species *A. vaga* and *Philodina roseola* also exhibit extreme resistance to ionizing radiation (Gladyshev& Meselson., 2008). They are able to reassemble into large DNA blocks a genome shattered into pieces by multiple DSBs resulting from exposure to doses of more than 1000 Gy of gamma radiation, and survive those damages (Gladyshev & Arhipova, 2010). By analogy with *D. radiodurans*, it has been hypothesized that the extraordinary radiation resistance of bdelloid rotifers was an adaptation to survive desiccation in their characteristic 'terrestrial' habitats (Gladyshev & Meselson., 2008). However, no experimental evidence that desiccation causes DNA DSBs in bdelloids has been obtained so far. Interest in this question was renewed when the transcriptome of *A. ricciae* published by Boschetti et al. (2012) and the genome structure of *A. vaga* (Flot et al., 2013) revealed high frequencies of gene conversion events and of horizontal gene transfers (HGT), suggesting that the desiccation experienced by bdelloid rotifers in their natural temporary habitat may have reshaped their genome over time. Notably, the gene conversion inferred to occur between homologous regions, and the genomic rearrangements, could be the result of DNA DSB repair through mitotic recombination following desiccation. Also, during desiccation the integrity of membranes, including the gut lining, could become compromised and therefore facilitate the passage of ingested foreign DNA from the gut to the oocytes residing next to the digestive system. The exogenous DNA can then be integrated into the germ-line DNA, either through homologous or ectopic recombination during DNA repair, and be duplicated as suggested by Gladyshev & Arkhipova (2010). Such mechanisms occurring repeatedly over drought periods could account for the 8% of genes that bear strong signatures of non-metazoan origin and are likely to originate from HGT (Flot et al., 2013).

In order to tackle these intriguing hypotheses, we documented the genomic integrity of *A. vaga* individuals submitted to prolonged desiccation. We first ruled out that DNA DSBs occur

only in dead animals, as well as the possibility that animals accumulating DSBs were not able to recover upon rehydration and were therefore promised to a certain death. Knowing this, we demonstrate for the first time that desiccation does induce DNA DSBs in these metazoans, and that these damages are repaired after rehydration. We also studied the effect of UV-A and UV-B radiation on genome integrity of desiccated *A. vaga* individuals, in order to look at the influence of natural conditions on dried animals, and revealed an increase of DNA breaks with UV exposure.

Material and methods

The question asked (whether bdelloid rotifers accumulate DNA DSBs during desiccation without reducing their survival significantly) necessitates showing that i) DSBs accumulate during desiccation, ii) DSB accumulation take place in live animals and not only dead ones, iii) live animals with DSBs within their genome can survive desiccation upon rehydration and that survival was not simply limited to animals that did not experience damage, iiii) upon rehydration DNA DSBs are repaired. To answer these points, we: i) used Pulsed Field Gel Electrophoresis (PFGE) to measure the integrity of the genome during desiccation, ii) then measured and compared kinetics of DSB apparition in a population of live and dead desiccated animals, iii) genomes of all animals of a desiccated population were shuttered into small fragments using proton irradiation and survival rates were measured iiii) and finally repair kinetics were conducted. Below the methods used to reach these goals are outlined.

Bdelloid rotifer culture

All experiments were performed using isogenic *Adineta vaga* clones descending from a single individual from the Meselson Laboratory (Van Doninck et al., 2009). The cultures were maintained hydrated in 150 x 20 mm Petri dishes supplemented with natural spring water

144 (Spa®), at 16°C, and fed with *E. coli* 1655MG. To avoid bacterial proliferation and dust
145 accumulation, water was changed twice a month.

146 *Desiccation and survival rates*

147 Dense cultures of *A. vaga* individuals were starved for two days and then washed with Spa®
148 water to remove debris and dead animals. Individuals were detached from the bottom of the
149 Petri dishes using cell scrapers, collected in a 15 mL Falcon tube and centrifuged for 15 min
150 at 5000 rpm. The supernatant was removed and individuals were pooled and resuspended in
151 filtered Spa® water. 250 µl of this dense suspension, containing 10.000 to 15.000 *A. vaga*
152 individuals, were deposited in the center of a 3% Low Melting Point (LMP) agarose plate and
153 complemented with 1mL Spa® water. These Petri dishes were placed without lid in a climatic
154 WEKK 0028 chamber (Voetsch). The following desiccation conditions were applied: linear
155 decrease of relative humidity from 70% to 55% in 17 hours, linear decrease of relative
156 humidity from 55% to 41% during 1 hour, followed by 19 hours at 41% relative humidity.
157 After these 37 hours all the water had evaporated, the LMP layer was dry and the bdelloids
158 appeared desiccated. Dehydrated *A. vaga* individuals were maintained at 41% RH and 23°C
159 during 1, 7, 14, 21, 42 and 84 days of desiccation. Four desiccation plates were used for each
160 time point (1 for PFGE analysis and 3 plates measuring survival rates).

161 To determine the survival rate after desiccation, Petri dishes were removed from the climatic
162 chamber at specific intervals (see below) and rehydrated with 15 ml Spa® water. The survival
163 rate after desiccation was estimated by counting living and dead individuals 24 hours after
164 rehydration. Bdelloids were considered alive when the mastax moved in contracted
165 individuals or if they had fully recovered motility.

166 Group effect on survival rate was tested by varying the amount of *A. vaga* individuals (1, 10,
167 50, 100, 500, 1000 or 5000 individuals per plate) in the desiccation protocol while

maintaining the final volume at 1.25 mL water. These plates were transferred to a non-hermetic room at 20°C where relative humidity was maintained around 40% (+/- 12%) using a Frigor FDH 12 dehumidificator. Desiccated plates were kept dry during 14 days in this specific experiment.

Measuring the residual moisture content.

In order to confirm complete desiccation of *A. vaga* clusters, the water content of hydrated and desiccated (1 and 7 days) *A. vaga* clusters was determined using a thermogravimetric analysis (TGA) following an adapted protocol from Alcazar et al. (2000). Each tested condition was done in duplicate. TGA measures the amount of weight change of a material in function of a temperature gradient and therefore detects residual water of dried samples. Desiccated *A. vaga* individuals were scraped from their LMP agarose support with a surgical blade. Hydrated specimens were detached from the bottom of the Petri dishes using cell scrapers, collected in a Falcon tube, centrifuged 15 min at 5000 rpm, re-suspended in 2 ml distilled water and centrifuged 3 times in order to remove impurities in the supernatant and all excess water. Desiccated and hydrated samples were placed in a measuring pan of a thermogravimetric analyser (Perkin-Elmer TGA 4000). The initial weight of the sample was determined, then under a constant nitrogen flow of 55 ml/min, starting at 30°C with a heating rate of 10°C/min, the temperature was raised to 60°C and maintained there for 100 min. The temperature was then raised to 125°C and maintained for 7 min in order to complete the water elimination and determine the weight or dry mass of the sample (free water residual mass). Finally, temperature was raised to 600°C with a heating rate of 5°C/min to examine thermal decomposition of the sample.

Genomic DNA integrity

Genomic DNA integrity was assessed using PFGE. Petri dishes were removed from the climatic chamber at specific intervals (see below) and rehydrated with a cold solution of 50 mM EDTA 10 mM Tris (pH 8.0). One thousand contracted, desiccated *A. vaga* individuals were harvested and resuspended in 25 µL of cold 50 mM EDTA 10 mM Tris (pH 8.0), then mixed with 25 µL of 1% low melting point agarose (LMPA; NuSieve GTG) freshly melted in a buffer containing 200mM EDTA, 100 mM Tris (pH 8.0) and finally casted in a plug mold at 42°C. After 15 min of polymerization, plugs were individually transferred into 500µL digestion buffer (100mM EDTA 50mM Tris, pH8, supplemented with 1mg/mL proteinase K (Fermentas) and 1% N-Lauroylsarcosine sodium solution), kept for one hour at 4°C and then incubated 18 h at 56°C. As a control, we used plugs containing *S. cerevisiae* chromosomes (BioRad) treated with the same digestion buffer. Plugs were then incubated in 1mL 0.5X TBE at 4°C for 3h, rinsed with 0.5X TBE then kept in 1mL EDTA 0.5M (pH 8.0) at 4°C until use. The complete lysis of the bdelloid individuals embedded within the plugs was controlled visually with a microscope. Plugs were loaded in a gel (0.8% LMPA, 0.5X TBE) and migration was performed using a BioRad CHEF-DR II instrument (14°C; 5.5V/cm, switch angle of 120° and switch times of 60-185 sec for 22h with a linear ramp). The PFGE gel was labeled in SYBR Gold (Invitrogen) and scanned with a BioRad Chemidoc XRS camera. ImageLab 3.0 quantification software (SybrGold settings) and ImageJ were used to process the images. Intact or slightly degraded chromosomes are expected to remain in the well, given the size of the *A. vaga* genome being ~ 244 Mb for 12 chromosomes (Flot et al., 2013; Mark Welch JL et al., 2004). DNA segments migrating within the resolution size of the PFGE (~<2,2 kb) are therefore resulting from important chromosomal degradation.

Killing A. vaga *individuals*

In order to follow DNA degradation in *A. vaga* individuals killed before entering into desiccation, the animals were desiccated following the same desiccation protocol but with

1.25 mL Spa® water supplemented with neomycin (Sigma) to a final concentration of 350 µg/mL. This dose is lethal to *A. vaga* and all individuals died right before complete desiccation. Both the dead and living *A. vaga* individuals were maintained during 1, 21 and 42 days in the same dried conditions.

Proton irradiation of desiccated A. vaga

A. vaga individuals desiccated for one day were submitted to 1.7 MeV proton radiation (delivering 25keV/µm) using Tandetron 2 MV (Wera et al. 2008; 2011). Dose rate was equal to 10 Gy/min. Survival rate after radiation was investigated as for the desiccation protocol. Number of DNA DSBs induced by proton radiation was calculated based on *A. vaga* genome size, 240 Mb (Flot et al., 2013), and average molecular size observed after doses of 500 Gy and 800 Gy.

DNA repair kinetic

Two repair kinetics were performed on *A. vaga*. First, we studied the DNA repair of DSB damage induced by 21 days of desiccation. Second, *A. vaga* individuals desiccated for one day were exposed to 800 Gy of proton radiation and DNA repair was investigated. In both experiments, genomic integrity was checked on surviving individuals after 0, 2, 8, 24 and 48 h of rehydration. PFGE setup was as described above.

UV-A and UV-B radiations

A. vaga individuals desiccated for one day were submitted to UV-B radiation (wavelength of 312 nm) generated by three TL 20 W/01 lamps (Philips, Eindhoven) placed 30 cm above the rotifer individuals. The precise dose of radiation emitted was measured using a UVR radiometer Vilber VLX3W with UV-B sensor (312 nm). The same protocol was performed

for UV-A radiation using three Philips 40W Cleo Performance lamps emitting at broad range of UV-A (310-400mm with maximum intensity at 360 nm). Radiations were recorded using the Vilber VLX3W radiometer with UV-A captor (360 nm). Covering the petri dishes with dark paper blocking 100% of the UV-A and UV-B radiations assessed a potential heat effect on survival rates.

Results

Survival rates after desiccation are strongly influenced by "cluster formation"

To address the impact of desiccation on the survival of *A. vaga*, we measured the survival rate of various numbers of *A. vaga* individuals after 14 days of desiccation. The rate appeared strongly dependent on the number of bdelloid individuals present in the drop of water deposited on the desiccation medium (Figure 1a): while one isolated individual died 100% of the time, batches of 5,000 individuals presented a ~ 93% survival rate. In this latter condition, dead individuals were isolated on the plate, whereas living *A. vaga* individuals emerged from clusters. Therefore, increasing the number of bdelloids involved in a desiccation experiment enhances both the probability to form dense clusters and the survival rate. This observation suggests that upon slow desiccation bdelloid rotifers tend to group and form clusters as a natural strategy to escape death. In controlled conditions set to 23°C, 41% +/-1% RH, clusters formed by 10.000 to 15.000 *A. vaga* individuals exhibited a survival rate at 14 days of 98% (Fig. 1b), and still as high as 75% after 84 days of desiccation. For subsequent desiccation experiments and survival rate evaluation, only *A. vaga* individuals present in desiccated clusters with a minimum of 10.000 individuals were considered. A thermogravimetric analysis was performed on dried *A. vaga* clusters to confirm complete desiccation of the whole cluster. Water content of hydrated *A. vaga* rotifers was estimated at 94% and decreased

to a maximum residual water content of 6,5% of dry weight in 1 and 7 days desiccated *A. vaga* clusters (supp. data 1) as observed by Lapinski & Tunnacliffe (2003) for dried bdelloids.

DNA breaks accumulate during desiccation

The integrity of chromosomes during desiccation was investigated through PFGE. First, in order to test for the influence of the experimental procedure on the generation of DNA DSBs, we treated chromosomes of *S. cerevisiae* embedded in agarose plugs similarly to the plugs containing rotifers. No sign of degradation was observed, suggesting that the lysis *per se* does not generate DNA DSBs (Fig. 2a, lane 9). The genome of *A. vaga* comprises 12 chromosomes of ~20kb, and therefore *A. vaga* full-length chromosomes do not match the resolution of the PFGE (between 225 to 2200 kb) and will remain in the wells. As expected, the control genomic DNA of 1000 hydrated *A. vaga* individuals was observed to remain in the gel plug, with a weak signal around 2 Mb (Fig. 2a, 2b, 2c, lane 2). No DNA smear was detected in *A. vaga* individuals desiccated for one day, although a slight increase of DNA fragments > 2 Mb was observed (Fig. 2a, lane 3). After 7 days of desiccation a well-defined smear appeared, reflecting the apparition and migration into the gel of DNA fragments ranging in size between 225 and 2,200 kb (Fig. 2a, lane 4). The amount of chromosomal fragments migrating into the gel kept increasing as a function of the time spent in desiccated state (Fig. 2a). Therefore, although the drying process *per se* does not appear to generate significant amount of DSBs (see lane 3 on Fig 2a: 1 day completely desiccated), a prolonged period in desiccated state results in the accumulation of DNA DSBs in the bdelloid rotifer *A. vaga*.

Together, these two experiments also reveal that the influence of "group" formation onto survival rate is not directly linked to protection against DNA DSB and/or increased repair efficiency.

Survival to desiccation is not related to genome integrity

DNA DSBs were observed after 7 days or more in desiccated state (Fig. 2a) while the survival rate remained high (>75%; Fig. 1b). The apparition of degraded DNA in our PFGE (Figure 2a) could eventually solely reflect the accumulation of DSBs in the genomes of the few dead animals present in the cluster and not in those of living rotifers. To address this issue, we first tested whether an increase in DNA damages was observed in 1,000 dead *A. vaga* individuals submitted to desiccation, as compared to living ones. Death was induced by a lethal dose of neomycin just before entering desiccation. If DNA DSBs occurred because of "death", we expected to observe a significant, precocious increase in DSBs in the dead group compared to the control, since both were composed of the same number of individuals. After 21 or 42 days of desiccation, the accumulation of DNA DSBs in the group of living individuals (with < 15% mortality) was similar, both in timing and amount, to the one in the group of dead individuals (Fig. 2b). This result confirmed that DNA DSBs accumulate during desiccation independently of the animal condition (dead or "alive"). The accumulation of DNA DSBs observed on Fig. 2a is therefore the consequence of desiccation and not of mortality.

Then, we investigated whether the survival of desiccated *A. vaga* individuals was dependent on the amount of DSBs present in the genome. To do so, we measured the survival rate of one-day desiccated *A. vaga* individuals exposed to high dosage of ionizing radiation (proton radiation, doses from 0 Gy to 800 Gy; Fig. 1c and 2c; M&M). Proton radiations generate DSBs through direct interaction with the DNA molecule, in opposition to gamma radiations that induce damages indirectly through the generation of reactive oxygen species from water molecules (Gusev et al., 2010; S. Lucas pers comm). Proton radiations are therefore well-suited here to induce breaks in dried rotifers. Fifty Gy of proton radiations was sufficient to induce DSBs and DNA fragments clearly visible on the gel (Fig. 2c, lane 4). With average fragment sizes of 450 and 365 kb for respective exposition to 500 Gy and 800 Gy (Fig. 2c, lanes 7 and 8) an estimation of 0,004 DSB Gy^{-1} MB^{-1} is calculated (based on genome size of

244 Mbp; M&M). Despite these highly fragmented genomes in desiccated state, the survival rate of *A. vaga* was not affected and reached 99% (Fig. 1c), a rate similar to non-irradiated *A. vaga* individuals desiccated for one day (Fig. 1b). This result demonstrated that the accumulation of DNA DSBs in desiccated *A. vaga* individuals does not alter significantly survival rate, and that *A. vaga* are able to recover from massive DNA damages upon its exit from the desiccated state.

DNA Repair kinetic

To investigate whether the genomes of dried *A. vaga* are efficiently repaired at the exit of the desiccation process, we analyzed the DNA repair kinetics of individuals desiccated for 21 days and subsequently rehydrated for 48 hours. Restoration of complete mobility of rehydrated *A. vaga* was observed between 2 to 24 h after rehydration. As expected, DNA damages were observed in animals just rehydrated (Fig. 3a, lane 3). Then, the smear of small DNA fragments (< 2,200 kb) progressively vanishes during the 48 hours spent in the rehydrated state (Figure 3a). Similarly, the amount of large (≥ 2,200 kb) DNA segments also gradually decrease in the gel, revealing the presence of an active DNA DSB repair mechanism after desiccation. Interestingly, after 48 h of rehydration, large DNA fragments (≥ 2 Mb) remained present in the genomes of fully mobile individuals.

We then tested further the ability of dried individuals to cope with massive DNA DSBs by recording DNA repair of rehydrated individuals that were one-day desiccated and submitted to 800 Gy of proton radiations. Interestingly, rehydrated *A. vaga* were able to repair these damages (Figure 3b): they gradually reduced the amount of small DNA fragments (225 -1125 kb) of their shattered genomes and regained larger DNA fragments over time. Despite full recovery of mobility 8h after rehydration, DNA damages were still observed in 48h rehydrated *A. vaga*.

Overall, these results demonstrate that rehydrated *A. vaga* individuals submitted to desiccation are able to handle extreme numbers of DNA DSBs, and underlie the presence of a remarkably efficient DSB repair mechanism. In addition, we also show that the exit of the desiccated state is not impaired by the presence of DNA breaks, and that these animals recover motility and metabolic activity.

Effect of UV-radiations on survival and genome integrity

UV-A and UV-B radiation, in contrary to proton and gamma radiation, are radiations commonly encountered in natural habitats. Whether such wavelengths are likely to affect the survival and the genome integrity of dried bdelloids remains unknown. We asked whether the combined effect of desiccation with UV exposure (other than the harsh proton irradiation performed in laboratory conditions) would also have an effect on the amount of DNA DSBs. To test this, *A. vaga* individuals desiccated for one day were exposed to increasing doses of ultraviolet radiation (50, 100 and 150 $kJ.m^{-2}$). Surprisingly, exposure to UV-A or monochromatic UV-B radiations decreased the survival rate on the contrary to proton radiations (see Fig. 1d). UV-B appeared systematically more harmful than UV-A: 10% of dried *A. vaga* survived a dose of 150 kJ UV-B while 38% survived the same dose of UV-A (Fig. 1d). Both UV-A and UV-B induced DNA damage: a smear was observed at each tested dose on PFGE (Fig. 4). UV-B induced a higher amount of damage than UV-A with fragments above 2 Mb still being observed at the highest UV-A dose of 150 $kJ.m^{-2}$. An increase in DNA degradation was observed for both UV-A and UV-B following a dose-dependent curve (Fig. 4a and 4b respectively). In comparison with UV-treated desiccated individuals, no DNA damage was observed for those protected from UV rays but experiencing the heating effect of the UV lamp (Fig. 4, lanes 5, 7 and 9 on both gels). Overall, these results suggest that UV is an important source of DNA DSBs in desiccated animals, and that the damages generated by this type of radiations are more harmful to *A. vaga* rotifers than proton radiation. Seeking

protection from these electromagnetic radiations is therefore crucial for rotifers entering the desiccation stage.

Discussion

Desiccation in the bdelloid rotifer Adineta vaga

In the present study, we refined the experimental conditions needed to promote desiccation of adult bdelloid rotifers with optimal survival (i.e. through a controlled, progressive evaporation of water over 37 hours on a LMP substrate). Interestingly, we observed that the ability to survive desiccation was highly correlated with the bdelloids aggregating together. This aggregation phenomenon had already been observed in desiccation-resistant tardigrades and nematodes, and was described as a way to reduce the evaporation rate by reducing exposed body surface area (Ivarsson 2004). Clusters of *A. vaga* individuals were already present 15h through the dehydration process, when agarose was still hydrated. Given that dead individuals did not reorganize into clusters, such aggregation appears as a dynamic response of *A. vaga* individuals upon increasing hydric stress. After 37h of slow dehydration, LMP agarose with clustered *A. vaga* individuals were completely dry as determined by the thermogravimetric analysis. According to the literature, complete desiccation is reached when water content decreases below 10% of dried mass. At this level there is no longer enough water to form a monolayer around macromolecules, preventing enzymatic reactions and therefore metabolism (Billi and Potts, 2002; Alpert 2005).

In the nineteenth century, Davis (1873) was the first scientist to study the desiccation mechanism in bdelloid rotifers. He proposed that in addition to body contraction, the gelatinous fluid secreted around the body of bdelloid rotifers is a key factor for desiccation survival. Clusters of desiccated bdelloids therefore appear to be capped in a “gelatinous

385 varnish" which may enhance protection. The nature of this substance is still unknown and it
386 remains an open question, as well as the influence of the clustering on the ability of bdelloid
387 rotifers to resist environmental stresses in general.

388 *Impact of desiccation on genomic integrity*

389 Although the causality between desiccation and resistance to radiation-induced DNA damages
390 in bdelloid rotifers has been regularly invoked, it was never demonstrated experimentally
391 (Gladyshev& Meselson, 2008). Here we show that indeed the genome of dried *A. vaga*
392 individuals is accumulating DNA DSBs as a function of time spent in desiccated state. The
393 genomic integrity was equally impacted in living and dead dried *A. vaga* individuals,
394 suggesting that DNA repair is not an active process during desiccation and that cells of *A.*
395 *vaga* experiment a suspended metabolic state.

396 Entering a desiccated state is generally accompanied by a metabolic shutdown and the
397 generation of reactive oxygen species (ROS) known to promote DNA damage if not
398 neutralized by antioxidants (França et al 2007, Gusev et al 2010). Interestingly, recent data
399 suggest that strong antioxidant machineries appear to be present in *A. vaga* protecting its
400 cellular components from oxidative damage during exposure to high doses of ionizing
401 radiation (Krisko et al., 2012). This was corroborated by genomic data indicating that gene
402 families involved in resistance to oxidation have significantly expanded in this lineage (Flot et
403 al., 2013). Such an arsenal of antioxidants may have been selected over time to protect
404 proteins from oxidative damage during desiccation, including those involved in DNA DSB
405 repair.

406 *Effect of DSB accumulation on genomic content*

407 We show that the survival rate is equivalent (and almost 100%) for non-irradiated one-day
408 desiccated bdelloids and dried animals submitted to 800 Gy proton radiations (carrying more

than 700 DNA DSBs per cell). Our results confirm the extraordinary radiation resistance of dried *A. vaga*, as observed by Gladyshev et al. (2008) on hydrated individuals. Proton radiations are targeting all tissues without discrimination; it is therefore very unlikely that the observed DNA DSBs in desiccated *A. vaga* are restricted to somatic cells only. A previous study by Gladyshev & Arkhipova (2010) demonstrated that DNA damage induced by gamma radiation upon multiple *A. vaga* generations resulted in the loss of a mariner-like *Avmar1* transposon copy. This result confirmed the presence of DNA DSB damage in oocytes that are subsequently repaired and are potentially responsible of the elimination of transposable elements. Indeed, during homologous recombination repair, ectopic crossing over between dispersed transposable elements can result in lethal chromosomal rearrangements, selecting against a high TE content (Gladyshov & Arkhipova, 2010). By analogy with these observations, desiccation cycles observed in the natural environment may be the source of DNA DSBs needed to purge TEs from this genome unable to undergo conventional meiosis (Flot et al. 2013). Indeed the genome of *A. vaga* contains only about 3% of transposable elements, which is much less than the percentage found in other metazoans (Flot et al., 2013). Besides the DNA DSBs induced by desiccation, RNA-mediated silencing machineries may contribute to the prevention of TE expansion in *A. vaga* (see Flot et al., 2013).

Recovery from desiccation: DNA repair kinetic

The massive amount of DNA damage induced by high doses of radiation (Fig. 2c) is not preventing the recovery process of *A. vaga*, neither in hydrated (Gladyshev et al., 2008) nor in desiccated state. Our rehydration kinetics shows that full mobility recovery is reached after 8h of rehydration despite the presence of DNA damages and that DNA repair is active upon rehydration since we observe a decrease in small DNA fragments. At 48h DSBs were still present but less. This is consistent with data collected in the chironomid species *P. verplanki* where the genome integrity was retrieved only after 96h of repair (Gusev 2010). Fisher et al.

(2013) also found that the repair of cyclobutane-pyrimidine dimers (CPDs) induced by UV-B radiation on hydrated bdelloid *Philodina roseola* was almost complete at 96h. Moreover, in this latter study they demonstrated that UV-B damage was only repaired when *P. roseola* went through a desiccated state. Desiccation therefore appears a key factor to induce DNA repair in bdelloids (Fischer et al., 2013).

How desiccated animals avoid apoptotic or necrotic processes that should normally be induced by multiple DNA DSBs remains unknown and how organisms with a genome shattered into small pieces can reassemble fully functional chromosome(s) also remains an open question. When it comes to *A. vaga* an interesting hypothesis regarding repair is coming directly from the genome structure described in Flot et al. (2013): the observed degenerate tetraploidy could promote efficient DNA repair since homologous regions are useful for template-dependent repair of DSBs as well as insure that, within the reservoir of gene copies, at least one will remain intact after desiccation. The presence of intact copies of essential genes may explain why during the recovery stage most individuals appear fully motile.

UV-A and UV-B are modulating genomic integrity of desiccated A.vaga

It has been demonstrated that environmental conditions such as temperature, relative humidity or oxygen have an impact on the genome integrity of dried organisms (for instance on bacteria, Yang et al., 2009) and on DNA (Bonnet et al., 2010). Recently, Hall et al. (2014) studied the impact of environmental UV radiation on dried DNA and found that DNA DSBs and oxidative lesions are the main damages induced.

In the present study, we tested the impact of UV-A and UV-B radiation on one-day desiccated *A. vaga* and show that both UV types promote DNA DSBs (Fig. 4). Corroborating with our results, UV-A and UV-B were also recently reported as sources of DNA DSBs in forensic samples (Hall et al., 2014) or desiccated *Bacillus subtilis* (Moeller et al., 2007). We also

found that UV-A and UV-B induced damages were significantly affecting the survival rate of desiccated *A. vaga* individuals in contrast with protonic radiation induced damages (Fig. 1C, 1D). The resulting decrease in survival rate may be linked to non-reversible oxidative damage affecting key proteins involved in metabolism restoration since UV radiation also induces considerable oxidative lesions (Altiero et al., 2011). In their natural environments, such as mosses and lichens, *A. vaga* individuals experiencing desiccation are likely to be exposed to solar UV radiations. In some specific conditions, *A. vaga* can be exposed to massive UV irradiation, for instance in Arctic or Alpine mosses (Kaya et al., 2010; Fontaneto & Melone, 2003), or when carried by wind at high altitudes (under the form of contracted "tun"; Wilson & Sherman 2013). Besides such extreme conditions, it is interesting to outline that the relatively mild UV-A dose used in this study was able to significantly impair *A. vaga* survival during desiccation. The dose used here appears in the same range of those experienced by wild bdelloid populations exposed directly to sunlight in Belgium: on October 1st 2013 we recorded doses of 45 kJ/m^2 UV-B and 200 kJ/m^2 UV-A during 7h direct exposure to sunlight. Therefore, current results suggest that solar radiation might play an important role in modulating the genomic integrity of desiccated bdelloids and their survival; since bdelloid rotifers are asexual, one individual is sufficient to start a new population. A predictable outcome is that important bottleneck events may reshape continuously *A. vaga* populations, and together with desiccation may contribute to rapid speciation events in this clade.

Evolutionary consequences of desiccation on an ameiotic evolution

Living in temporary habitats, *A. vaga* individuals probably undergo multiple cycles of desiccation during their life cycle. It has been hypothesized that desiccation-induced DNA DSBs shape bdelloid genomes and promote gene conversion through mitotic recombination during DNA DSB repair (Flot et al., 2013). The desiccation process also appears to be a key mechanism to repair DNA damage, as it was observed in hydrated *P. roseola* individuals

where DNA damage induced by UV-B was only efficiently repaired after a round of desiccation (Fischer et al., 2013). The interplay between the genome structure and DNA repair of DSBs following desiccation events remains to be deciphered. Interestingly, a degenerated Spo11 gene has been characterized in the genome of *A. vaga* although conventional meiosis is not expected, and alternative roles of proteins usually involved in meiotic pathways may also be interesting to investigate (Hörandl & Hadace 2013). Finally, repetition of desiccation events over time associated with frequent DSBs may favor the integration of horizontally transferred genetic material, accounting for 8% of the gene content of *A. vaga* that appears to be of non-metazoan origin (Flot et al., 2013). This challenging but exciting hypothesis remains to be tested, but if true would provide a remarkable example of genomic adaptation to a natural environment as has been observed in plant-parasitic nematodes (Paganini et al., 2012).

The clustering of *A. vaga* individuals during the dehydration process was positively linked with survival rate. In nature, *A. vaga* individuals regroup before entering into desiccation and are therefore more likely to increase genetic exchange between them. We have currently no empirical data supporting genetic exchange between bdelloids. However, it seems likely that, given the fact that the *A. vaga* genome contains 8% of horizontally acquired genes from non-metazoans, exchange among bdelloids occur. The formation of DNA DSBs in desiccated bdelloids is a plausible gateway to asexuality by promoting integration of horizontally transferred genetic material during drought and rehydration cycles.

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Author Contributions

B.H., M.K. and D.H.M. maintained the rotifer cultures. B.H. performed all experiments with help of M.K. for the group effect study and D.H.M. and M.K. contributed to the optimization of the desiccation protocol and PFGE. A-C.H. and B.H. performed the proton exposures, supervised by S.L. TGA were performed by F. P and B.H. The results were analyzed and the manuscript written by B.H., R.K. and K.V.D. The project was designed and supervised by R.K. and K.V.D. and the acquisition of funding was done by K.V.D.

FIGURE LEGENDS

Figure 1. Effect of group, time and radiation on the survival rate of desiccated *Adineta vaga* individuals. (a) Survival rate of *Adineta vaga* as a function of the number of individuals during 14 days of desiccation (Material and Methods) (**b**) Effect of time spent in desiccated state on the survival rate of *A. vaga* 24h after rehydration. (**c**). Effect of proton radiation on the survival rate of *A. vaga* who spent one day in desiccated state (Material and Methods). (**d**) Effect of UV-A (diamonds) and UV-B (squares) radiation on the survival rate of *A. vaga* who spent one day in desiccated state. Standard deviations are represented by error bars.

Figure 2. Genome integrity of desiccated *Adineta vaga*. The three panels show PFGE. The first and last lanes on the PFGE correspond to the karyotype of *Saccharomyces cerevisiae* without and with treatment to lysis buffer. Other lanes correspond to 1,000 *A. vaga* individuals submitted to desiccation and/or radiation. The control on each gel corresponds to 1,000 hydrated *A. vaga* individuals. (**a**) Effect of time (1, 7, 14, 21, 42 and 84 days) spent in desiccated conditions on the apparition of DNA DSBs. (**b**) Apparition of DNA DSBs during the time spent in dry conditions (1, 21 or 42 days) in living (d+) and dead (d-) animals. (**c**) Effect of proton irradiation (in gray Gy) on the apparition of DNA DSBs in one-day desiccated animals.

Figure 3. (**a**). Repair kinetic of rehydrated *A. vaga* after 21 days of desiccation. (**b**). Repair kinetic of rehydrated *A. vaga* after one day of desiccation with exposure to 800 Gy proton radiation. The first and last lanes on the PFGE correspond to the karyotype of *S. cerevisiae* without and with treatment with lysis buffer. Second lanes correspond respectively to 21 days desiccated bdelloids and one day desiccated bdelloids submitted to 800 Gy proton radiation. Other lanes correspond to 1,000 desiccated *A. vaga* individuals after 2, 4, 8, 24 and 48 hours of rehydration.

Figure 4. PFGE analysis of the effect of UV-A (**a**) and UV-B (**b**) on the genome integrity of one-day desiccated *A. vaga* individuals. The first lane on the PFGE corresponds to the karyotype of *S. cerevisiae*. Other lanes correspond to 1,000 *A. vaga* individuals desiccated for one day and exposed to UV for 50, 100 or 150KJ. For each irradiation experiment, 1,000 *A. vaga* individuals were exposed to the same temperature (Ctl t°) for the same amount of time presented.

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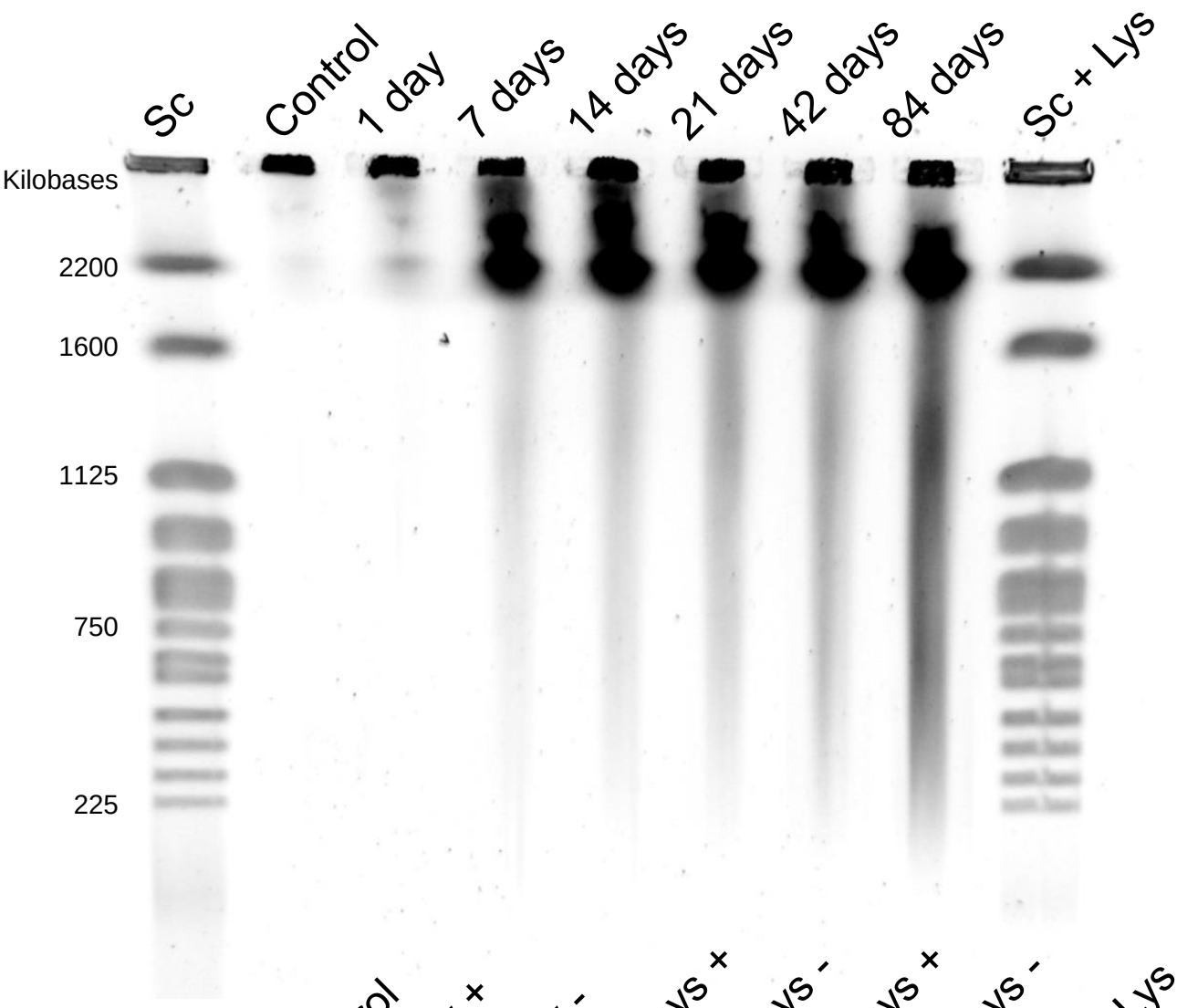
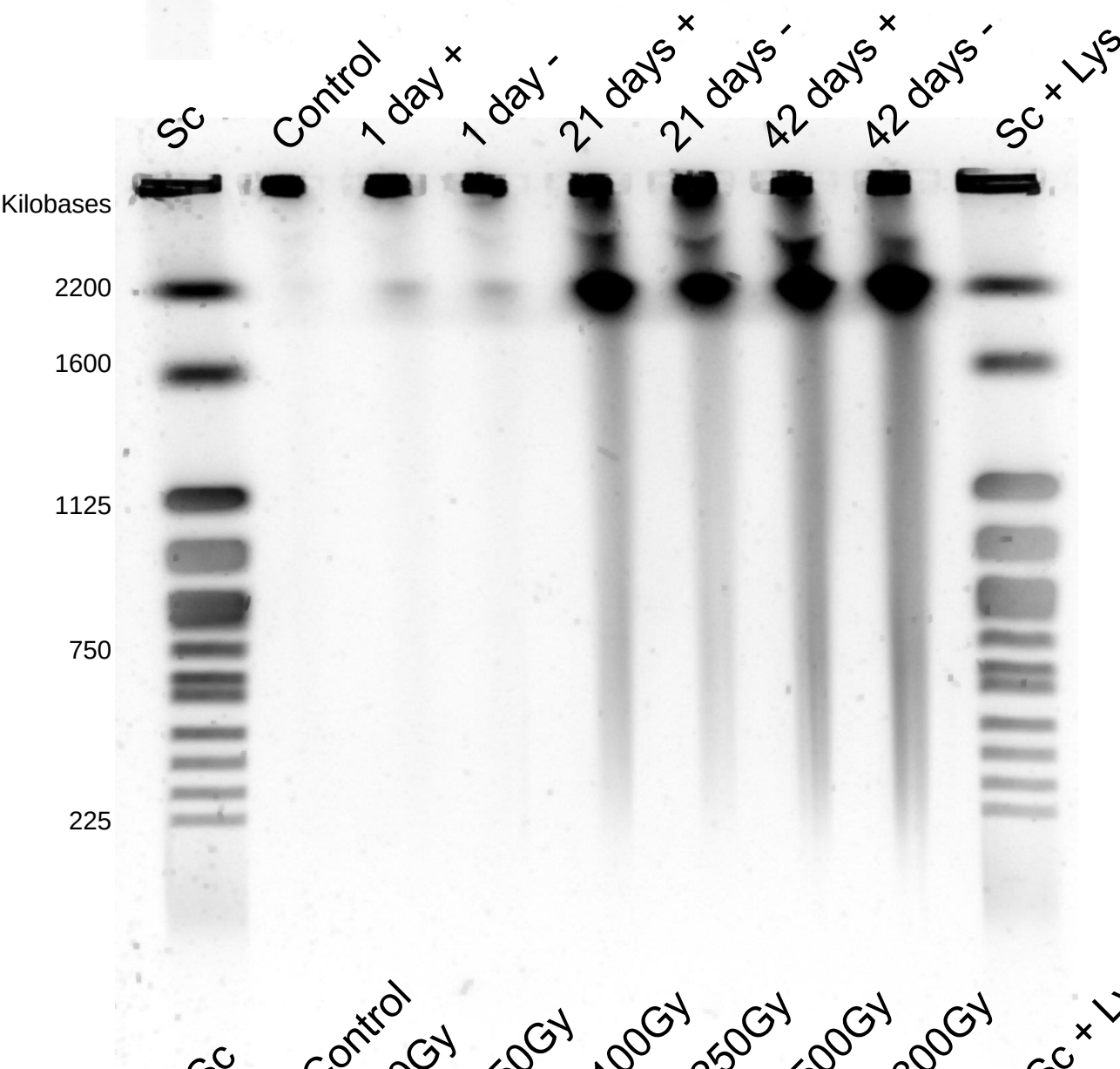
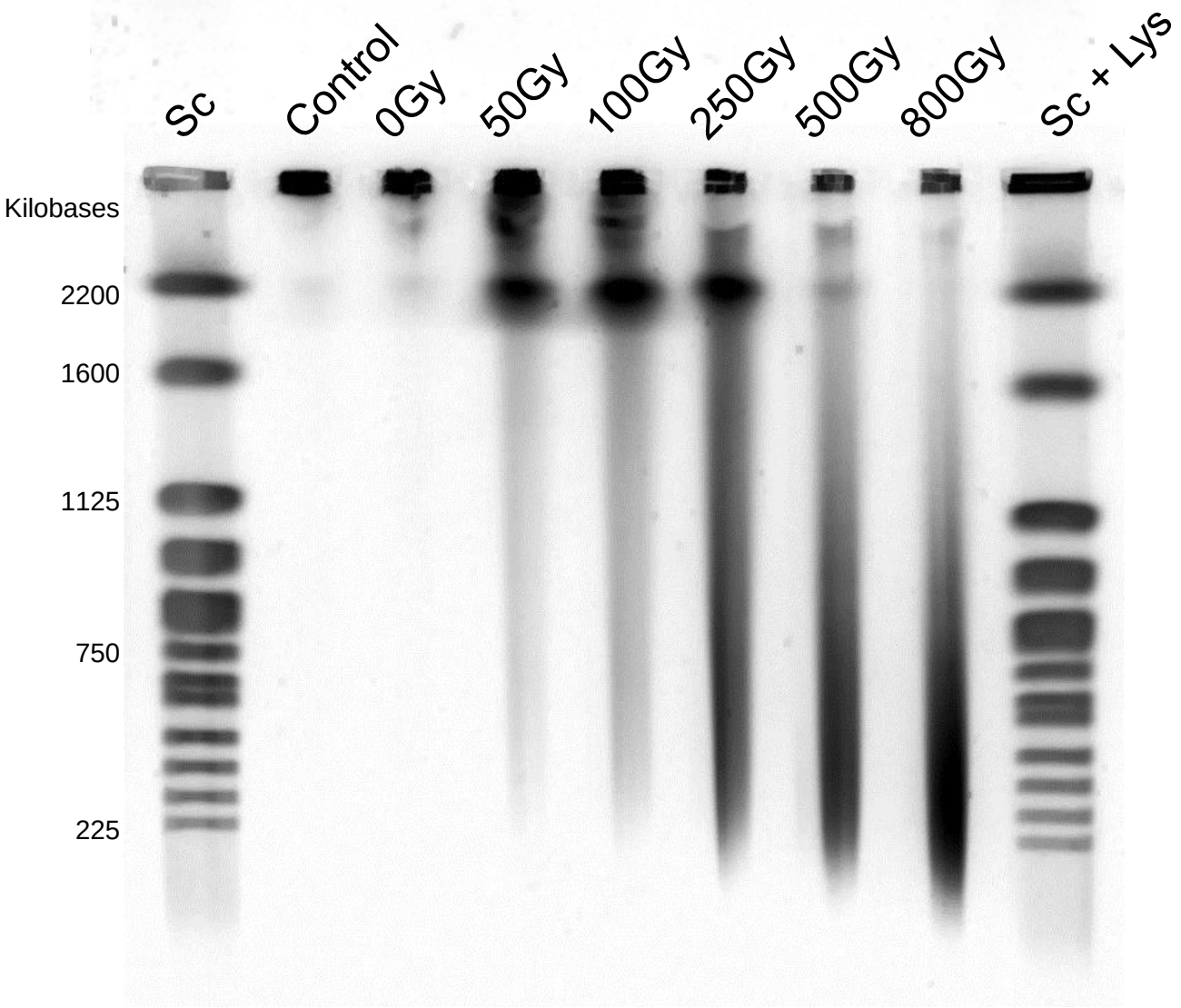
(a)


Sc
Control
1 day
7 days
14 days
21 days
42 days
84 days
Sc + Lys
Kilobases
2200
1600
1125
750
225

(b)


Sc
Control
1 day +
1 day -
21 days +
21 days -
42 days +
42 days -
Sc + Lys
Kilobases
2200
1600
1125
750
225

(c)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100Gy
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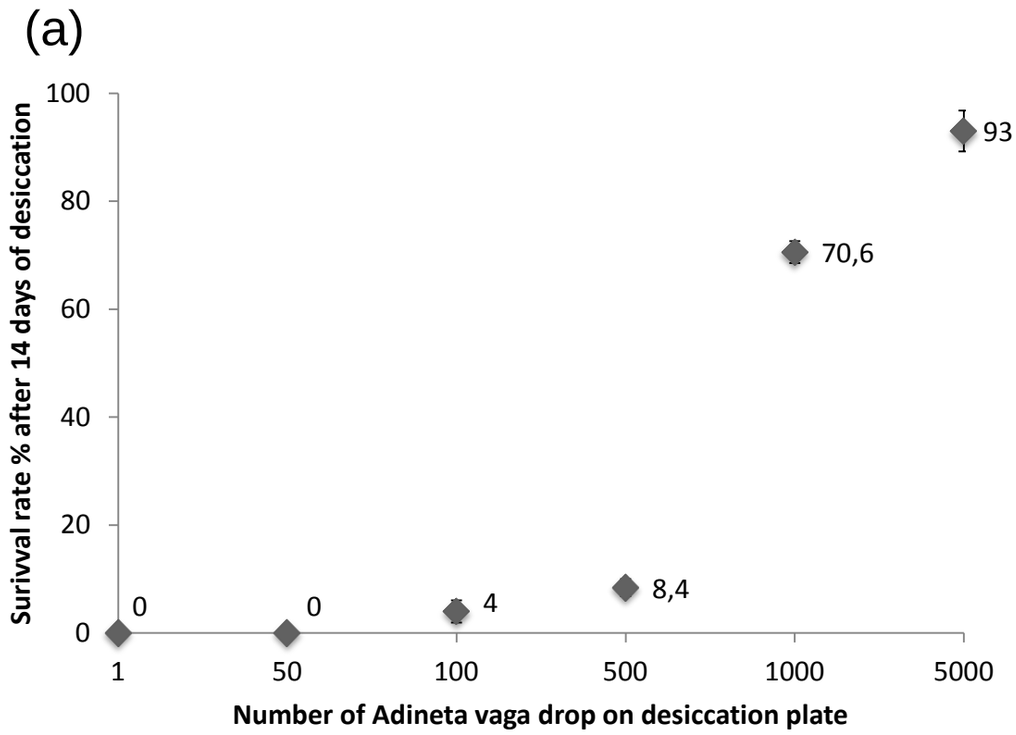
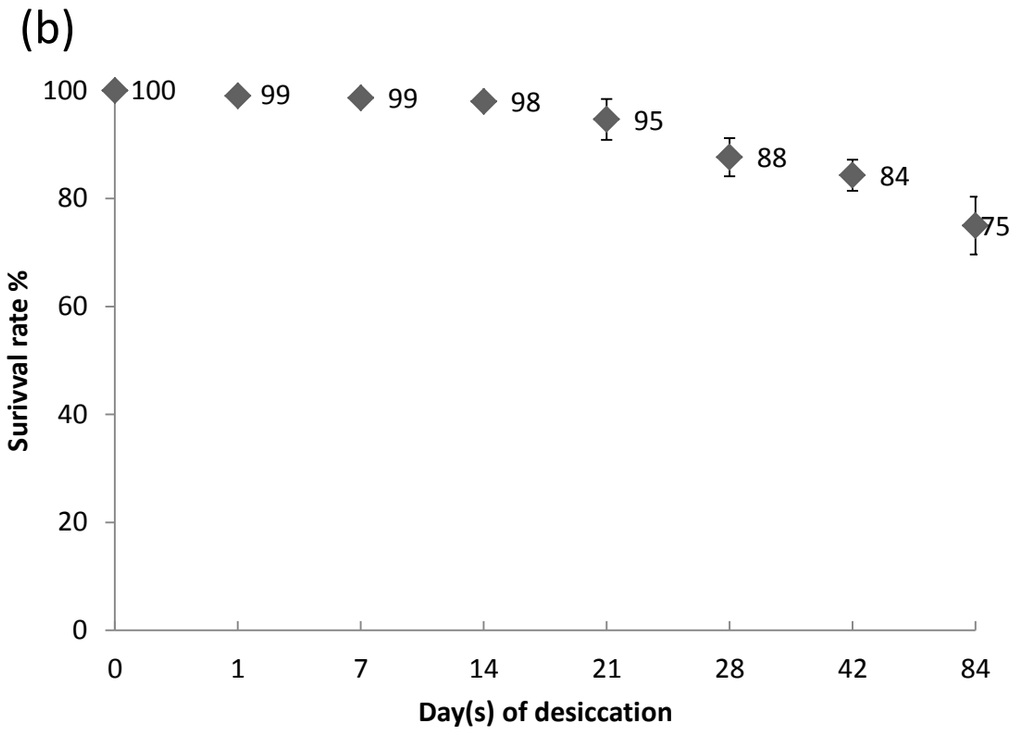
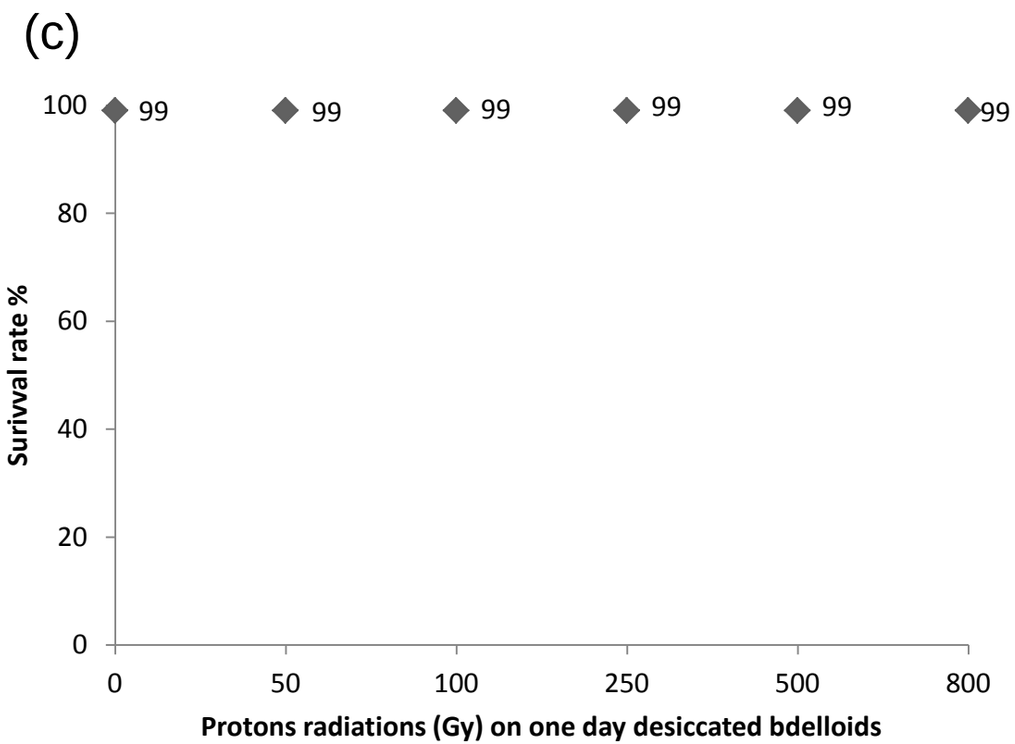
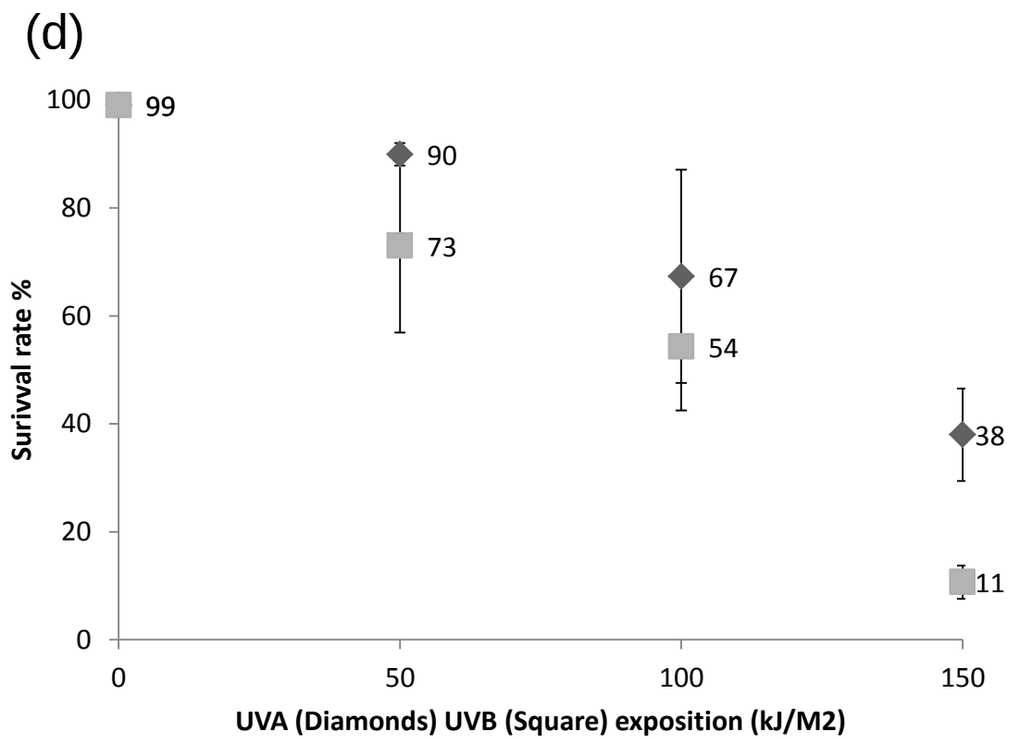
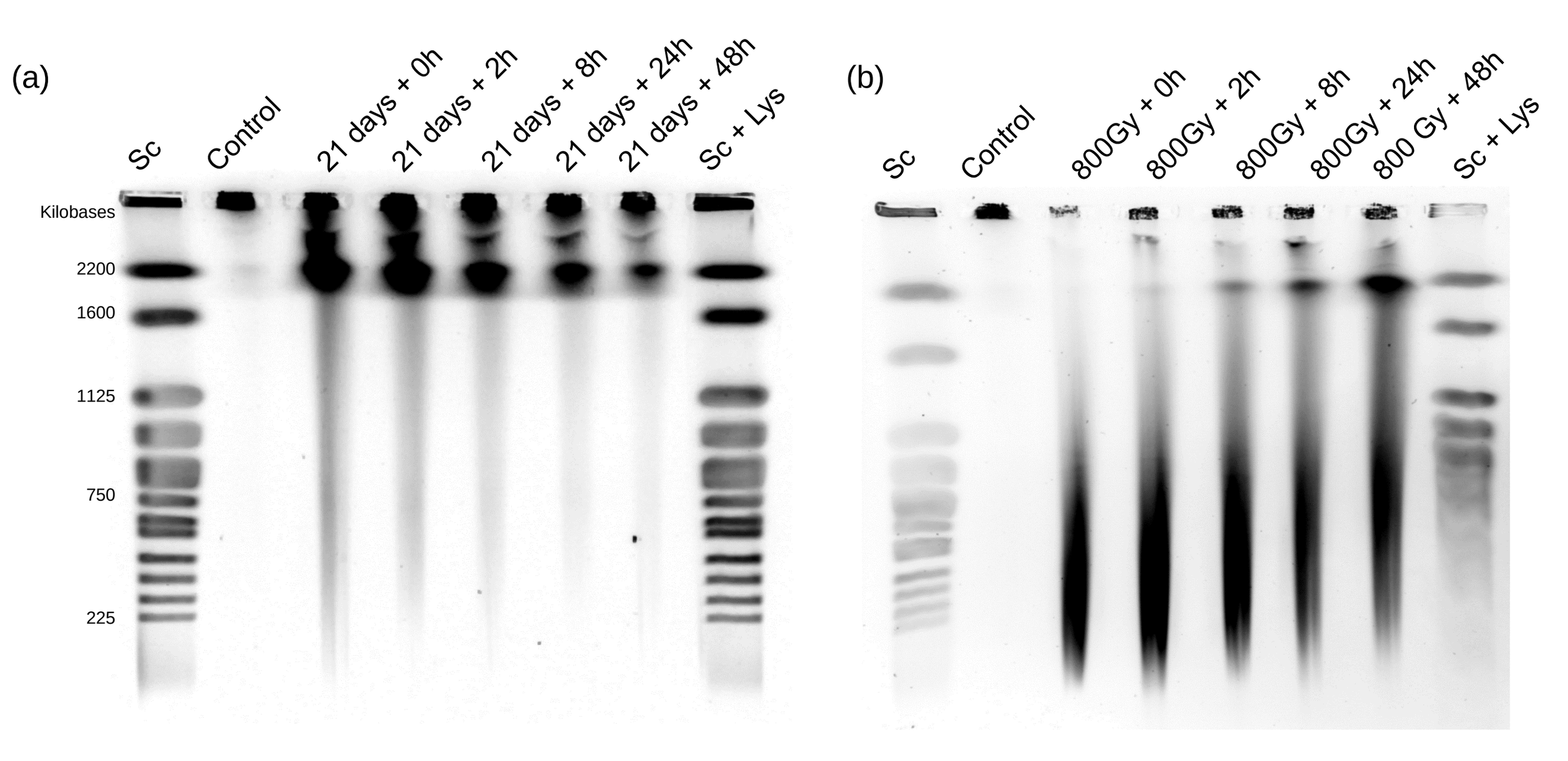
Sc + Lys
Kilobases
2200
1600
1125
750
225

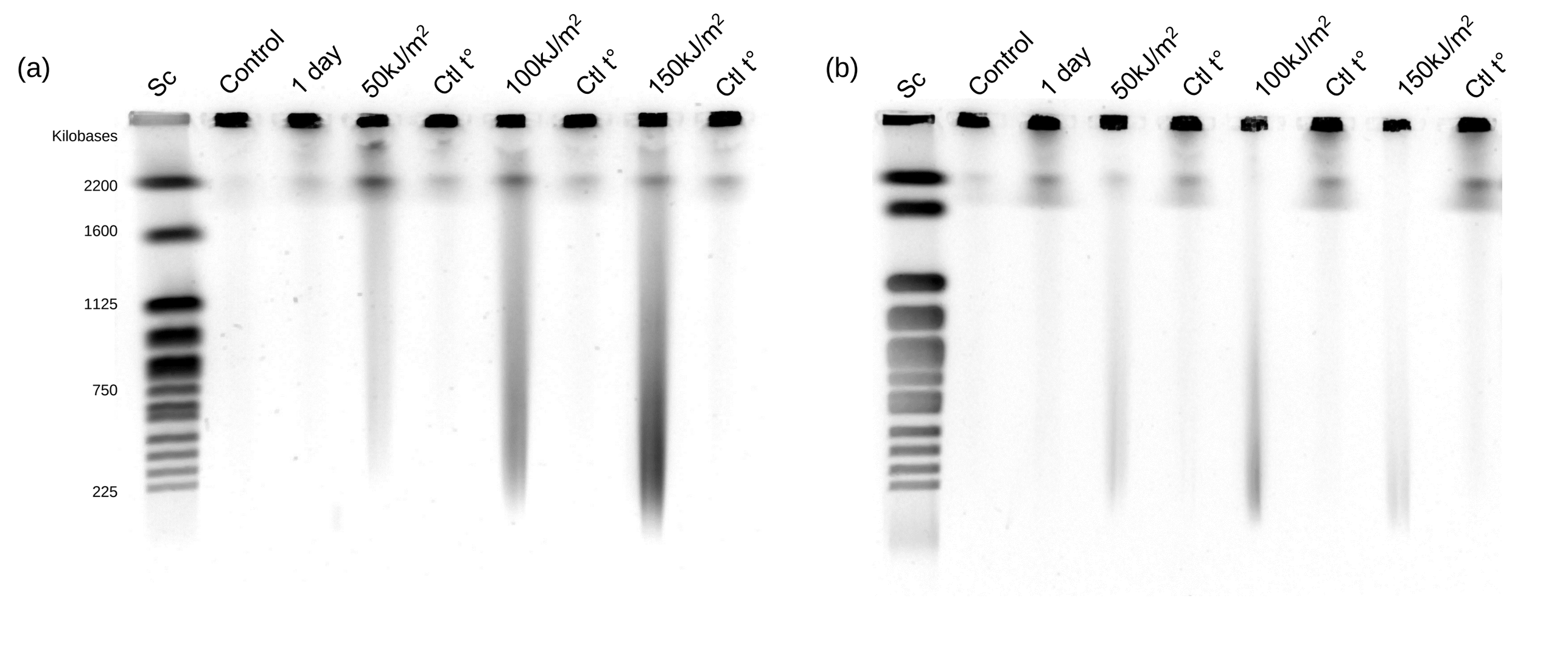

(a)
Surivval rate % after 14 days of desiccation
100
80
60
40
20
0
0
0
4
8,4
70,6
93
1
50
100
500
1000
5000
Number of Adineta vaga drop on desiccation plate


(b)
Surivval rate %
100
80
60
40
20
0
100
99
99
98
95
88
84
75
0
1
7
14
21
28
42
84
Day(s) of desiccation


(c)
Surivval rate %
100
80
60
40
20
0
99
99
99
99
99
99
0
50
100
250
500
800
Protons radiations (Gy) on one day desiccated bdelloids


(d)
Surivval rate %
100
80
60
40
20
0
99
90
73
67
54
38
11
0
50
100
150
UVA (Diamonds) UVB (Square) exposition (kJ/M2)


(a)
Sc
Control
21 days + 0h
21 days + 2h
21 days + 8h
21 days + 24h
21 days + 48h
Sc + Lys
Kilobases
2200
1600
1125
750
225
(b)
Sc
Control
800Gy + 0h
800Gy + 2h
800Gy + 8h
800Gy + 24h
800 Gy + 48h
Sc + Lys


(a)
Sc
Control
1 day
50kJ/m2
Ctl t°
100kJ/m2
Ctl t°
150kJ/m2
Ctl t°
Kilobases
2200
1600
1125
750
225
(b)
Sc
Control
1 day
50kJ/m2
Ctl t°
100kJ/m2
Ctl t°
150kJ/m2
Ctl t°