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Heterogeneous relationships between abundance of soil surface invertebrates and radiation from Chernobyl

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ABSTRACT

Although pollution due to nuclear accidents constitutes some of the largest environmental disasters, there is surprisingly little information available on the relationship between abundance of animals and background radiation. We sampled invertebrates with pit-fall traps at Chernobyl in areas differing in background radiation level by more than four orders of magnitude. We obtained samples from 58 traps for 19 taxa of which five showed positive associations (Acari, Araneae, *Formica* sp., Homoptera), while four showed negative associations (Coleoptera, Collembola, *Vespa*, Insecta). These relationships were independent of other environmental factors such as habitat, humidity and ecotype. Estimates of the relationship between abundance and background radiation from the pitfall trap study were similar to those from an independent study based on invertebrates found under slices of wood placed on the ground. The differences in relationship between abundance and radiation among taxa may arise from direct effects of radiation. The findings reported here have implications for choice of animal taxa for efficient monitoring of the biological impact of radiation on animals.

1. Introduction

Radioactive material occurs under natural conditions due to the radionuclides contained in rocks and from cosmic rays and such radioactive hotspots show significant negative effects on plants and animals (review in Møller and Mousseau, 2013a,b). Radioactive contamination due to release from nuclear disasters has affected hundreds of thousands of square kilometers across the globe with particularly strong effects having been documented at Chernobyl and Fukushima. Studies have shown that the abundance of organisms is affected by radioactive contaminants. This applies to soil invertebrates (Krivolutski and Pokarzhevsky, 1992; Krivolutski et al., 1999; Maksimova, 2005), bacteria (Zymenko et al., 1995; Romanovskaya et al., 1998; Ragon et al., 2011), spiders (Møller and Mousseau, 2009, 2011; Møller et al., 2012b,c), insects (Møller and Mousseau, 2009, 2011; Møller et al., 2012b,c), birds (Møller and Mousseau, 2007a,b; Møller et al., 2012b,c) and mammals (Møller and Mousseau, 2013a). Such effects of radiation on abundance are consistent among years and geographical locations (Møller et al., 2011, 2012b), but differ among taxa.

There are significant differences among bird species in susceptibility to radiation in Chernobyl with species on average being negatively affected in terms of abundance, while a few species show no or even a positive effect (Møller and Mousseau, 2007b). Similar heterogeneity has been documented for the relationship between abundance and level of background radiation in Fukushima (Møller et al., 2012b,c). While such heterogeneity in effects of radiation on abundance may be expected simply for sampling reasons, statistically significant heterogeneity beyond sampling suggests that these are inherent species-specific characteristics. Indeed, interspecific differences for birds in frequency of cataracts (Mousseau et al., 2013), reductions in brain size (Møller et al., 2013), frequency of tumors and abnormalities (Møller et al., 2013), aspermy and sperm behavior (Møller et al., 2008, 2014) and levels of antioxidants (Bonisoli-Alquati et al., 2011; Galván et al., 2014) suggest that underlying physiological processes account for such heterogeneity.

Although soil invertebrates, arachnids and insects constitute significant parts of biodiversity, and although small invertebrates are readily sampled in standard pitfall traps, there are so far no pitfall trap studies conducted in Chernobyl or Fukushima. While

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decomposition of leaf litter is reduced in contaminated areas around Chernobyl (Mousseau et al., 2014), these effects are not readily interpreted because we do not know to which extent different soil-inhabiting taxa are affected.

The main objective of this study was to assess to what extent the abundance of soil surface invertebrates was affected by radioactive contamination from Chernobyl. First, we sampled invertebrates with pitfall traps to test for differences in susceptibility to radiation among taxa. Second, we identified the taxa that provide the most sensitive indicators of radiation. Third, we cross-validated our estimates of effects of radiation on abundance by comparing our pitfall trap results with those obtained from invertebrates found under slices of pine wood placed across a radiation gradient.

2. Methods

2.1. Sampling sites

We chose six sites that were ecologically similar ranging in level of contamination from 0.013 to 36 mrem/h (Fig. 1). The coordinates of the sites and their characteristics are listed in the electronic supplementary material (Table S1). We report characteristics of these six study sites in Table S1 in terms of radioactivity, soil type, amount of dead organic matter, amount of surface covered with open water as in ponds, and dominant vegetation.

Soil at the traps was classified as sand or ash (ash derived from forest fires) within a radius of 10 m from each of the trap sites. The amount of litter was classified on a three-point scale from 1 – little, over 2 – common to 3 – abundant within a radius of 10 m from the traps. We recorded if there was open water (ponds or puddles) within a distance of 50 m from the traps. Finally, we recorded whether the dominant vegetation was grass, deciduous trees or coniferous trees within a distance of 10 m from the traps.

We considered three different ecotypes based on soil, abundance of litter and other features of habitats (Ingegnoli, 2002; Bastian et al., 2003).

2.2. Cross validation

We cut 5 cm slices from the trunks of four Scots pine (two from an uncontaminated area and two from a highly contaminated area) that were approximately 60 years old. At each of 20 sites varying in radioactive contamination by more than four orders of magnitude we put our one slice of each of the four trees in early June 2013. Each slice was identifiable from a unique numbered metal tag. During early June 2014 we re-visited all 20 sites and recorded on the lower surface facing toward the soil the number of fungi and the animal taxa used in the present study and counted these animals before turning over the slice again for future study. We recorded background radiation at the site of each slice when this was originally put out and one year later as described above. For the five taxa with animals common in the two datasets we estimated the abundance of animals in relation to background radiation using Poisson regression as described below.

2.3. Measuring background radiation levels

We measured radiation levels in the field at ground level at each location where a pitfall trap was deposited using a hand-held dosimeter (Model: Inspector, SE International, Inc., Summertown, TN, USA). We measured levels 2–3 times at each of these locations and averaged the measurements for each location. Such data were correlated with data from governmental measurements at ground level published by Shestopalov (1996), estimated as the mid-point of the ranges in the published maps. These analyses showed a high degree of consistency between the two methods (Møller and Mousseau, 2007a). Radiation levels vary by several orders of magnitude at a scale of 1 km due to heterogeneity in deposition of radioactive material after the Chernobyl accident (Shestopalov, 1996).

2.4. Pitfall traps and handling

We used 500 ml plastic containers with an inner diameter of 90 mm and 25% ethylene glycol as a preservative. They were placed in the ground so the top of the container was at the level of the soil surface and covered with CDs (compact discs) for protection from rain and solar light. The distance from the soil surface to the CD was about 2 cm. The distance among traps was 2 m, and a total of 20 traps per site we used in this survey. The traps were placed along two intersecting transects to form a “+” and oriented toward north, south, east and west. The traps were placed on 25 August 2007 and contents were collected on 8 September 2007.

2.5. Identification

Container contents were sorted under a stereo binocular microscope and transferred to 1.5 ml eppendorf tubes with 96% ethanol. The organisms were identified to order using Chinery (1993, 2007), Gibbons (1995), Gilarov (1964) and a mite identification web site (http://www.lucidcentral.org/keys/v3/mites/Invasive_Mite_Identification/).

Some samples were excluded from the analysis because they were destroyed by wild animals (the containers were dry or removed). One trap (site RF03) was excluded because it contained an enormous number of ants (667) and collembolas (26,922) that exceeded the general sample by more than three standard deviations. This aberrant trap was situated very close to an ants’ nest hence explaining the abundance of ants.

2.6. Statistics

Because observations of animals in pitfall traps basically are count data, they have to be treated as having a Poisson distribution. Therefore, we used generalized linear mixed models with the abundance of animals as the response variable and background radiation and the first two principal component scores reflecting environmental variables (Table S2). None of these components were statistically significant predictors and hence they were excluded from the final statistical models.

We reduced the five environmental variables using principal component analysis. This analysis was based on the correlation matrix for the five environmental variables using varimax rotation. The principal component analysis revealed two factors with eigenvalues above one accounting for 60% and 25% of the variance, respectively. The first factor was strongly positively correlated with soil and ash, dead organic matter, presence of water and ecotype. The second factor showed a positive loading for presence of trees and ecotype. We subsequently extracted principal component scores for the 58 pitfall trap sites and used these scores as additional predictor variables in the analyses. All analyses were made with JMP (SAS, 2012). We report means ± 1 SE.

3. Results

3.1. Summary statistics

We found animals of three orders of the class Arachnida, eleven orders (ten orders and one genus – Collembola) of Insecta and one order (Myriapoda) of the class Chilopoda (Table 1). In total, this study was based on 12,049 animals (Table 1) ranging in abundance

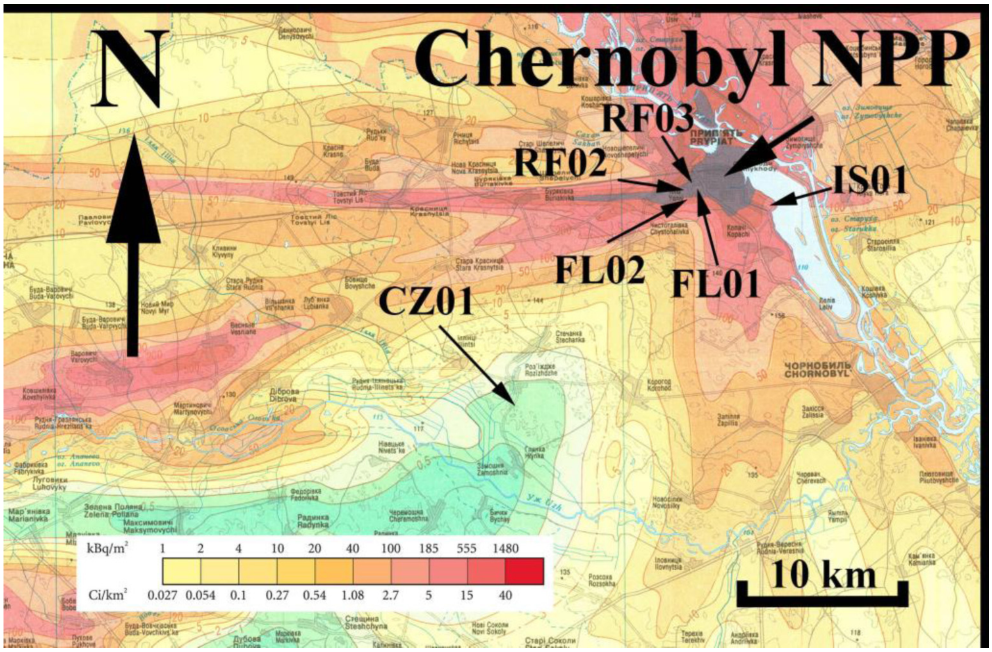

Fig. 1. Location of the six sampling sites for pitfall traps in the neighborhood of Chernobyl with background radiation indicated with increasing intensity of coloration (kBq/m^2). The large arrow shows the location of the Chernobyl Nuclear Power Plants.
Adapted from Shestopalov (1996). Further information on sites can be found in Table S1.

Table 1
Mean abundance, SE and total abundance of animals collected in 58 pitfall traps at Chernobyl.

Taxon	Taxon	Mean abundance	SE	Total abundance
Order	Araneae	3.71	0.34	215
Order	Opiliones	0.16	0.06	9
Order	Acari	48.81	3.70	2831
Class	Arachnida	52.67	3.69	3055
Order	Diptera	3.26	0.51	189
Order	Homoptera	0.33	0.15	19
Order	Orthoptera	0.43	0.10	25
Order	Hemiptera	4.67	0.67	271
Order	Lepidoptera	0.05	0.03	3
Order	Coleoptera	3.28	0.81	190
Order	Hymenoptera	13.29	1.52	771
Genera	Collembola	129.43	23.71	7507
Order	Thysanoptera	0.17	0.06	10
Order	Psocoptera	0.12	0.09	7
Order	Dermaptera	0.02	0.02	1
Class	Insecta	155.05	24.41	8993
Order	Myriapoda	0.02	0.02	1
Class	Chilopoda	0.02	0.02	1
Phylum	Arthropoda	207.74	25.79	12,049

from Chilopoda, Dermaptera and Myriapoda each with one individual to Collembola with 7507 and Acari with 2138 individuals.

3.2. Abundance and radiation

The abundance of animals depended on taxon, radiation and their interaction (Table 2). While there was no overall main effect of the relationship between abundance and radiation, the abundance of different taxa differed, and the interaction between taxon and radiation was highly significant (Table 2). This implies that at least one taxon differed from all others in relationship between abundance and radiation. Inspection of the data revealed that the relationship was negative for some taxa, positive for others and not significant for yet others (Fig. 2).

Five taxa showed an increase in abundance with radiation: Acari, Araneae, Arachnida, *Formica* sp. and Homoptera (Table 3 and Fig. 2). Three taxa showed a decrease with increasing radiation: Coleoptera, Collembola and *Vespa* sp. (Table 3 and Fig. 2). There was also an overall negative relationship for insects (Table 3).

3.3. Cross-validation of the relationship between abundance and radiation

We cross-validated the method used to estimate the relationship between abundance and radiation according to pitfall traps by comparing the estimates with an independent study based on invertebrates found under wood. The estimates of the relationship between abundance and radiation derived from the tree slice experiment were significantly positively related to the estimates based on pitfall traps for Acari, Chilopoda, Coleoptera, Collembola and *Formica* sp. ($F = 11.15$, $r^2 = 0.72$, d.f. = 1, 3, $P = 0.044$, estimate (SE) = 0.097 (0.029)). Likewise a non-parametric Kendall rank order correlation showed a significant positive relationship (Kendall $\tau = 1.00$, $P = 0.014$). Hence we conclude that the estimates of the relationship between abundance and radiation in different taxa were independent of the study method.

4. Discussion

The invertebrate taxa investigated here differed in their abundance relative to background radiation level with some taxa showing significant positive relationships, others significant negative relations and yet others no significant relationships. We could exclude the possibility that the findings were biased because the relationship between abundance and radiation was consistent in a comparison between estimates based on pitfall traps and estimates based on animals found under slices of pine wood deposited at 20 sites across a radiation gradient at Chernobyl. These findings imply that careful choice of specific taxa with a strong negative relationship between abundance and level of background radiation will provide the most sensitive assessment of the biological effects of radiation.

Table 2
Generalized linear mixed model of the relationship between abundance of animals and taxon, background radiation and their interaction. Values are χ^2 and associated *P*-values, estimates (SE) and 95% lower and upper confidence limits.

Term	χ^2	P	Estimate	SE	Lower CL	Upper CL
Intercept	6.89	0.009	−0.69	0.56	−2.36	−0.10
Taxon	42457.64	<0.0001				
Radiation	0.72	0.40	−0.21	0.31	−1.10	0.16
Taxon × Radiation	271.76	<0.0001				

There is a surprising deficit of census data from areas contaminated with radionuclides, and studies of soil invertebrates are restricted to only a handful (Krivolutski and Pokarzhevsky, 1992; Krivolutski et al., 1999; Maksimova, 2005; Møller and Mousseau, 2009, 2011; Møller et al., 2012b,c) despite the importance of these taxa for ecological processes. Here we have shown differences in the relationship between abundance of soil surface invertebrates and radiation, with Coleoptera, Collembola, *Vespa* sp. and insects showing significant negative relationships, Acari, Araneae, *Formica* sp. and Homoptera showing positive relationships and the remaining taxa showing no significant relationship. We can exclude the possibility that these differences were due to sampling alone because we were able to cross-validate our findings for five taxa (Acari, Chilopoda, Coleoptera, Collembola and *Formica* sp.) with data from another study that used a different methodology. Some of the relationships that we have documented here differed from those previously reported elsewhere. Thus Møller and Mousseau (2011) showed negative relationships between abundance and level of

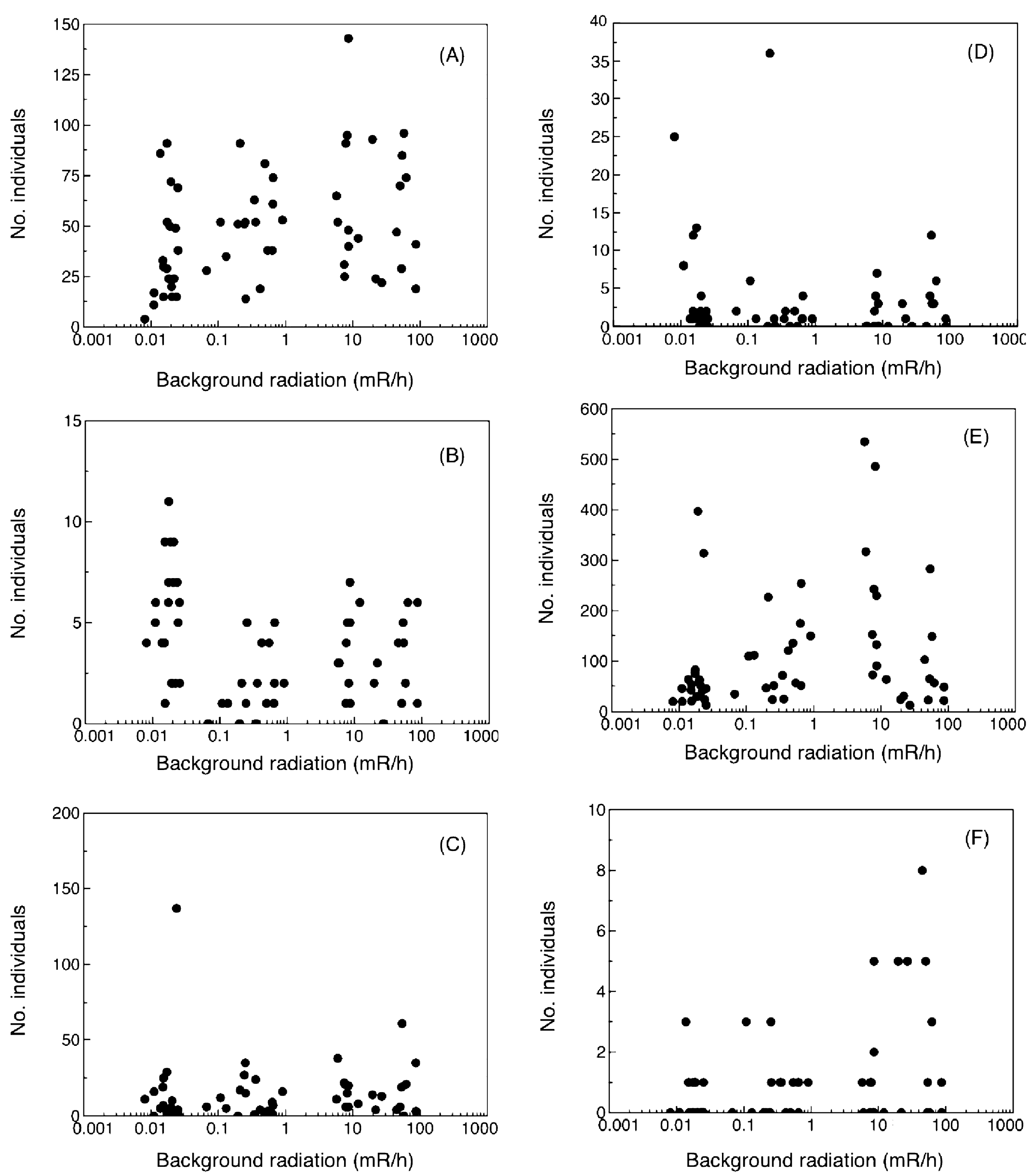

Fig. 2. Abundance of invertebrates in relation to background radiation (mR/h) at 58 sites near Chernobyl with pitfall traps. (A) Acari, (B) Araneae, (C) *Formica* sp., (D) Coleoptera, (E) Collembola and (F) *Vespa* sp.

Table 3
Results for the relationship between abundance and log-transformed background radiation from generalized linear mixed models with a Poisson distribution and log-link function for different taxa of animals at Chernobyl. Values are χ^2 and associated *P*-values, estimates (SE) and 95% lower and upper confidence limits.

Taxon	χ^2	*P*	Estimate	SE	Lower CL	Upper CL
Acari	9.28	0.0023	0.332	0.110	0.118	0.549
Arachnida	11.99	0.0005	0.365	0.106	0.157	0.575
Araneae	4.50	0.034	0.907	0.441	0.067	1.797
Chilopoda	0.31	0.580	4.570	8.740	−10.510	26.734
Coleoptera	10.64	0.0011	−1.289	0.391	−2.056	−0.518
Collembola	8.44	0.0037	−0.161	0.055	−0.270	−0.053
Dermaptera	2.65	0.103	71.000	106.100	−5.750	655.736
Diptera	0.71	0.400	−0.327	0.385	−1.068	0.443
Formica sp.	115.43	<0.0001	1.951	0.190	1.583	2.326
Hemiptera	0.25	0.610	0.154	0.308	−0.439	0.768
Homoptera	5.61	0.018	5.278	2.326	0.887	10.064
Hymnoptera	1.31	0.253	−0.202	0.176	−0.544	0.146
Lepidoptera	2.95	0.086	10.349	7.529	−1.053	30.457
Myriapoda	0.31	0.579	4.568	8.739	−10.510	26.735
Opiliones	0.00	0.995	−0.012	1.792	−3.477	3.923
Orthoptera	0.27	0.604	−0.614	1.171	−2.878	1.797
Psocoptera	0.19	0.668	0.928	2.216	−3.080	5.992
Thysanoptera	0.03	0.861	−0.370	2.099	−4.524	3.965
Vespa sp.	5.94	0.015	−1.579	0.627	−2.789	−0.318
Insecta	11.89	0.0006	−0.176	0.051	−0.276	−0.076

background radiation for spiderwebs, butterflies and grasshoppers, while here we did not find significant relationships for these taxa. The data are not comparable for Araneae because the previous study was based on web-building spiders, while most of the spiders in the present study were ground dwelling jumping spiders. Likewise, Møller and Mousseau (2011) analyzed diurnal butterflies while those analyzed here were mainly noctuid moths. Similarly, the Orthoptera analyzed by Møller and Mousseau (2011) were true grasshoppers, while those analyzed here were mainly crickets. Thus these data are not comparable.

Interpretation of the findings reported here will require detailed physiological and genetic analyses. The findings reported here may arise from differences in susceptibility to radiation, mutation rates, DNA repair or direct and indirect impact of radiation. For example, direct impact of radiation due to the toxic effects of radionuclides may result in damage to DNA or normal processes of physiological homeostasis. In contrast, indirect effects of radiation may act through effects on abundance of food, parasites, predators or competitors. There is currently very little empirical information on these possible mechanisms. For example, Beasley et al. (2012) showed for grasshoppers *Chorthippus albomarginatus* from Chernobyl in a common garden experiment that parental radiation exposure had little measurable effect on developmental instability in morphology, fecundity or survival. More studies of this sort are required for a better understanding of the underlying mechanisms responsible for differences in abundance of invertebrates related to differences in background radiation.

We measured background radiation using a handheld dosimeter. This approach rests on the assumption that the invertebrates investigated here are exposed to a similar level of radiation throughout their lives, and that their movements do not result in altered radiation exposure. This assumption seems likely for invertebrate taxa that do not move long distances, while it may apply less to taxa that disperse far such as spiders and butterflies. We have previously shown for different animal taxa and for birds that population density was more negatively affected by radiation when natal dispersal distance was long (Møller and Mousseau, 2011).

This study allows for a ranking of taxa with respect to their indicator ability of the biological effects of ionizing radiation in a similar way as Møller and Mousseau (2011) have done previously for a smaller number of invertebrates and for vertebrate taxa. The strongest positive effect was documented for ants of the genus *Formica* and Arachnida, while the strongest negative effect was documented for Coleoptera and Collembola.

In conclusion, invertebrate taxa sampled in pitfall traps differed in their susceptibility to background radiation with some taxa showing positive relationships between abundance and radiation, while others showed negative effects. Further experimental studies will be needed to unravel to mechanisms underlying this heterogeneity in population responses to environmental radiation exposures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2014.11.014.

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