GLOBAL CHANGE ECOLOGY - ORIGINAL RESEARCH

Ecosystems effects 25 years after Chernobyl: pollinators, fruit set and recruitment

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Abstract Animals are assumed to play a key role in ecosystem functioning through their effects on seed set, seed consumption, seed dispersal, and maintenance of plant communities. However, there are no studies investigating the consequences of animal scarcity on seed set, seed consumption and seed dispersal at large geographical scales. We exploited the unprecedented scarcity of pollinating bumblebees and butterflies in the vicinity of Chernobyl, Ukraine, linked to the effects of radiation on pollinator abundance, to test for effects of pollinator abundance on the ecosystem. There were considerably fewer pollinating insects in areas with high levels of radiation. Fruit trees and bushes (apple *Malus domestica*, pear *Pyrus communis*, rowan *Sorbus aucuparia*, wild rose *Rosa rugosa*, twistingwood *Viburnum lantana*, and European cranberry bush *Viburnum opulus*) that are all pollinated by insects produced fewer fruit in highly radioactively contaminated areas, partly linked to the local reduction in abundance of pollinators. This was the case even when controlling for the fact that fruit trees were generally smaller in more contaminated areas. Fruit-eating birds like thrushes and warblers that are known seed dispersers were less numerous in areas with lower fruit abundance, even after controlling for the effects of radiation, providing a direct link between radiation, pollinator abundance, fruit abundance and abundance of frugivores. Given that the Chernobyl disaster happened 25 years ago, one would predict reduced local recruitment of fruit trees if fruit set has been persistently depressed during that period; indeed, local recruitment was negatively related to the level of radiation and positively to the local level of fruit set. The patterns at the level of trees were replicated at the level of villages across the study site. This study provides the first large-scale study of the effects of a suppressed pollinator community on ecosystem functioning.

Keywords Chernobyl · Ecosystem functioning · Fruits · Plant recruitment · Radiation

Communicated by Jeff Karron.

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Introduction

The role of different taxa in the functioning of ecosystems is currently of great scientific and practical interest, given the looming extinction crisis due to human activity, including climate change (review in Hooper et al. 2005). The effects of key taxa on ecosystem functioning are widely assumed, based on microcosm experiments and small-scale field experiments (review in Loreau et al. 2002), although there is little empirical evidence derived from large-scale field studies.

Reduced abundance of pollinating insects like bumblebees and butterflies often impairs plant fertility and seed or fruit set because plant fecundity is frequently pollen-limited (Faegri and van der Pijl 1966; Proctor 1996). For

example, Knight et al. (2005) reviewed a large number of studies that showed evidence of pollen limitation. Burd (1994) reported that 62 % of 258 species showed evidence of pollen limitation. While pollen limitation may be important for seed set, this may not have consequences for seedling production or recruitment. In fact, evidence suggesting effects of pollen limitation on population dynamics of plants is meager at best (Ashman et al. 2004; Price et al. 2008; Waser et al. 2010). Because farming and forestry rely on pollination for maintaining high levels of productivity, pollination has been considered one of the most important ecosystem services (Costanza et al. 1997; Kearns et al. 1998). It is not surprising that there are no studies that have investigated these presumed effects of the absence or reduction in abundance of pollinating insects at large spatial scales because large-scale experiments are rarely if ever possible.

The Chernobyl disaster on 26 April 1986, which released vast amounts of radioactive material across large areas in northern Ukraine, southwestern Russia and southern Belarus, may provide such an example of the effects of absence of pollinators at a large spatial scale on reproductive performance of plants including local recruitment. This disaster may also have directly reduced the abundance of frugivores that are often the main dispersers of plant seeds. The ecological consequences of radiation from the Chernobyl catastrophe are poorly known because more than 20 years of research has so far failed to conduct even the most basic types of censuses of abundance and species richness (Zakharov and Krysanov 1996; Møller and Mousseau 2006; Yablokov et al. 2009). Only very recently have we shown dramatic reductions in species richness and abundance of breeding birds and major invertebrate taxa such as bumblebees, butterflies, grasshoppers, dragonflies, and web-building spiders around Chernobyl (Møller and Mousseau 2006, 2007a, 2011).

The objectives of this study were to test for a supposed effect of radiation on pollinator abundance at a large spatial scale, under the assumption that a lack of pollinators would have consequences for fruit production, abundance of frugivores, and recruitment by fruit trees and bushes. We did so by conducting extensive censuses of pollinators, frugivorous birds, and fruiting trees and shrubs across 19 villages in northern Ukraine differing in level of radiation by more than four orders of magnitude, with radiation varying by more than one order of magnitude within most sites.

Materials and methods

Study areas and censuses

This study was based on extensive censuses of pollinating insects, fruit set, and birds eating fruit within and just outside the Chernobyl Exclusion Zone in Ukraine with all villages used for sampling indicated in Fig. 1.

We censused pollinating insects during May–June 2006–2009, abundance of fruits and recruits of plants during September 2007, and birds eating fruit likewise during September 2007. Censuses were conducted at 19 villages separated by distances of up to 15 km (each village having been evacuated after the Chernobyl disaster in 1986). In each of these villages, we censused all fruit trees in all gardens, with most gardens in 2007 still holding at least one live apple and one live pear tree planted before 1986. At each village, we conducted point censuses, where an observation point constituted a location with 5 min of recordings of individual plants, their fruit, pollinators, and frugivorous birds.

Censuses of pollinating insects

A.P.M. conducted standard point counts during late May–early June 2006–2009 (a total of 898 counts widely distributed across forested areas), with each point being censused once during 5 min during which all bumblebees, butterflies, and birds seen or heard were recorded (Møller 1983; Bibby et al. 2005; Møller and Mousseau 2007a, 2009). We did not census other taxa such as Diptera because inclusion of additional taxa would have extended the duration of the study and hence increased exposure to radiation. Inclusion of Diptera and other insect orders would also have caused problems of distinguishing between pollinating and non-pollinating taxa. The bumblebees and butterflies recorded were all pollinators as shown by observations of these insects visiting flowers and having contact with reproductive parts of flowers during such visits. Honeybees *Apis mellifera* are now extremely rare in the Chernobyl Exclusion Zone, leaving insect pollination to species of wild bees, bumblebees, and butterflies. Indeed, our attempts to collect *Drosophila* from the Exclusion Zone in late summer 2002 were largely unsuccessful due to an almost complete lack of fruit, with the only exception being the town of Chernobyl where a study of honeybees using introduced bee-hives was locally associated with high fruit set for both apples and pears, but not in neighboring villages (our own unpublished observations). Møller and Mousseau (2009) report the identity of bumblebee and butterfly species for the first 3 years of the census of these insects. Census points were located at an interval of at least 100 m within forested areas around Chernobyl (Fig. 1). Forested areas were selected because censuses were conducted in order to study the effects of radiation in the main habitat in the Chernobyl Exclusion Zone, and because secondary growth in villages has now essentially turned these into secondary forest separated by open fields. The point counts were made across a range of

Fig. 1 The distribution of villages used for sampling in relation to radiation levels (*darker areas* have higher radiation levels) around Chernobyl Nuclear Power Plant (*NPP*) in Ukraine. The 19 villages used for the fruit census indicated by *filled circles*. Map redrawn from European Union (1998)
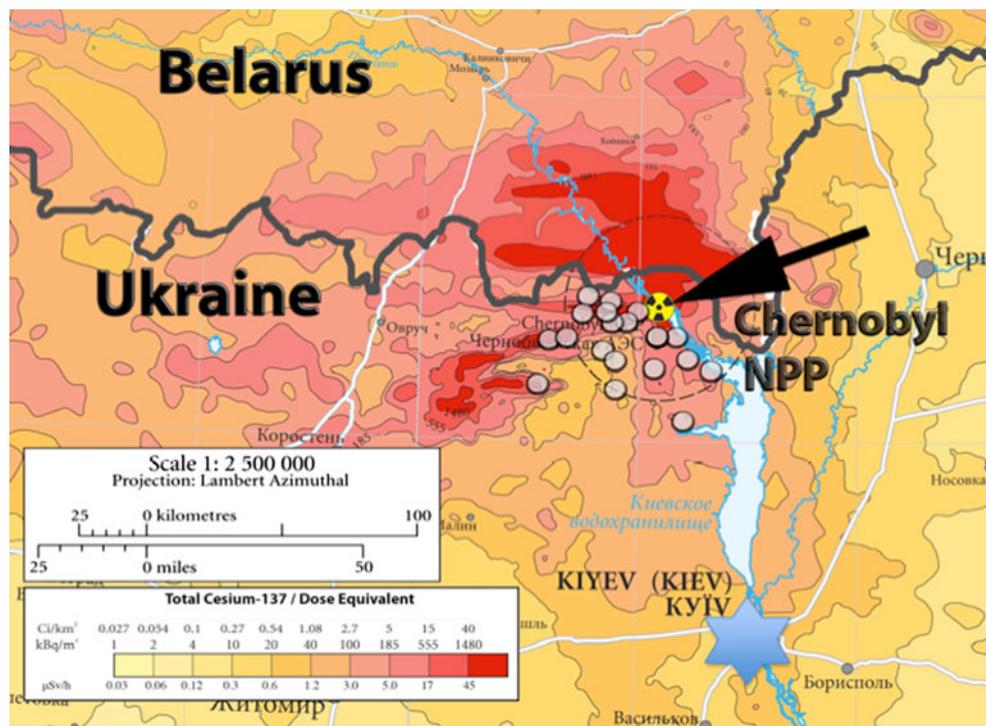
background radiation levels spanning over four orders of magnitude (Fig. 1; Møller and Mousseau 2007a, 2009).

Census of fruit bushes and trees

At each of the census points in September 2007m all fruit bushes and trees with a height larger than 1 m within a distance of 50 m from the location of the point were recorded using a pair of binoculars and inspection of all sites that were invisible from the census point. This amounted to a total of 1,329 bushes and trees of apple *Malus domestica*, wild pear *Pyrus communis*, wild rose *Rosa rugosa*, rowan *Sorbus aucuparia*, twistingwood *Viburnum lantanam* and European cranberry bush *V. opulus* across the same 374 census points that were used for censusing insects and birds. These six plant species constitute the majority of all widely distributed bird-dispersed plants, occurring commonly and widely across all study sites. All six species are generally self-incompatible and sexually reproducing with no vegetative or asexual propagation. These species are frequently visited by pollinators, and at least apple is known to require pollinators for maximum reproductive success (Mouton 2011). Although honeybees *Apis mellifera* are the most common pollinators of apples and pears (McGregor 1976; Stern et al. 2004; Mouton 2011), available data derive almost entirely from managed orchards with next to no information on pollinators under natural conditions. We used a height threshold of 1 m for inclusion because inclusion of shorter individuals would have required considerably more search effort

due to tall grass and shrub. We recorded height of all bushes and trees to the nearest meter because larger bushes and trees are expected to have more fruit, and radiation may reduce growth of bushes and trees. For a sub-sample of 30 bushes and treesm we subsequently measured actual tree heights with a measuring tape to the nearest meter. The two estimates were strongly positively correlated (Pearson $r = 0.98$, $t = 39.68$, $P < 0.001$), and did not differ significantly from each other (paired t test: $t = 0.57$, $df = 29$, $P = 0.57$). This provides evidence of our estimates of height of bushes and trees being reliable.

We also estimated the number of fruits on each bush or tree to the nearest multiple of ten. Again, for the same sub-sample of 30 bushes and trees mentioned above, we subsequently counted the exact number of fruits. The two estimates were strongly positively correlated (Pearson $r = 0.99$, $t = 221.73$, $P < 0.001$), and did not differ significantly from each other (paired t test: $t = 0.18$, $df = 29$, $P = 0.86$). This provides evidence of our estimates of the abundance of fruits being reliable.

Finally, we classified all apple and pear trees as recent recruits or older trees based on the clearly bimodal distribution of tree heights caused by recruitment from before 1986 having been prevented by human harvest of fruit. We set the height threshold for recent recruits that have entered the population since 1986 at 5 m. Among the 298 individuals 5 m or shorter, there were only two apple trees that were clearly older than the Chernobyl disaster, as judged from the diameter of their trunks, and that had recently had broken trunks causing these trees to be shorter than 5 m.

These two individuals were subsequently categorized as old trees pre-dating 1986, leaving 296 recruits. The results reported on recruitment rate were qualitatively similar, if we used a threshold of 4 or 6 m instead of 5 m.

Censuses of birds

A.P.M. censused birds including fruit-eating birds during September 2007 (a total of 374 points) using the same point census method as described above. Bird abundance estimates can be affected by many different potentially confounding variables (Møller 1983; Bibby et al. 2005), and, therefore, it is crucial to statistically control for the effects of such variables on abundance estimates. That must also be the case for the abundance of insects such as bumblebees and butterflies. We quantified habitat (agricultural habitats with grassland or shrub, deciduous forest, or coniferous forest estimated to the nearest 10 % of ground coverage within a distance of 50 m from each of the observation points). Maximum height of trees was estimated to the nearest 5 m, and soil type was classified as either loam/clay or sand. The presence or absence of open water within a distance of 50 m from each point was also recorded. Weather conditions can affect bird activity and hence census results (Møller 1983; Bibby et al. 2005), and it is well known that many bird are more active during sunny and warm weather than during cold and rainy conditions. Thus, we recorded cloud cover at the start of each point count (to the nearest eighth, range 0–1 during the censuses), temperature (degrees Celsius, range 12–28 °C), and wind force (Beaufort, range 0–4 during the censuses). For each point count we recorded time of day when the count was started (to the nearest minute). Because the activity of many birds shows a U-shaped relationship with time of day, with high levels of activity in the morning and less so in the evening (Møller 1983; Bibby et al. 2005), we also included time squared as an additional variable in the initial models. These environmental variables were entered into the initial statistical models to control for the potentially confounding effects of these variables (see "Statistical methods").

We considered the following bird species to be frugivores, based on our own observations in the field during the study (with the main fruits eaten mentioned in parentheses): blackbird *Turdus merula* (all species), song thrush *Turdus philomelos* (all species), fieldfare *Turdus pilaris* (all species), mistle thrush *Turdus viscivorus* (all species), robin *Erithacus rubecula* (*Viburnum*), redstart *Phoenicurus phoenicurus* (*Viburnum*), black redstart *Phoenicurus ochruros* (*Viburnum*), bullfinch *Pyrrhula pyrrhula* (*Sorbus*, *Viburnum*), blackcap *Sylvia atricapilla* (*Viburnum*), garden warbler *Sylvia borin* (*Viburnum*), and greenfinch *Carduelis chloris* (*Rosa rugosa*). A large fraction of the diet of these species consists of fruit during fall and winter. Our observations of frugivory by these species fit well with what is reported in the ornithological literature (Cramp and Perrins 1988–1994).

Measuring background radiation levels

We measured radiation in the field and cross-validated these measurements with those reported by the Ministry of Emergencies. Once having finished the 5-min census, we measured α, β, and γ radiation levels at ground level directly in the field at each point using a hand-held dosimeter (Model: Inspector; SE International, Summertown, TN, USA). We measured levels 2–3 times at each site and averaged the results. We cross-validated our measurements against data from the governmental measurements published by Shestopalov (1996), estimated as the mid-point of the ranges published. This analysis revealed a very strong positive relationship [linear regression on log–log transformed data: $F = 1{,}546.49$, $df = 1{,}252$, $r^2 = 0.86$, $P < 0.0001$, slope (SE) $= 1.28$ (0.10)], suggesting that our field estimates of radiation provided reliable measurements of levels of radiation among sites. The measurements by the Ministry of Emergencies were obtained by repeated standardized measurement of radiation at the ground level in a large number of different localities in Ukraine. Radiation levels vary considerably at geographical distances of hundreds of meters due to heterogeneity in the deposition of radiation after the Chernobyl accident (Fig. 1; Shestopalov 1996), and our measurements at the census points ranged from 0.01 to 379.70 μSv/h, or more than four orders of magnitude. Among the 19 villages, radiation levels varied by more than an order of magnitude in 12 of them. Thus, radiation level was a continuous variable in the analyses. The abundance of census points at different radiation levels is shown in the figures in Møller and Mousseau (2009).

Statistical methods

Abundance of fruit, tree height, abundance of pollinators and frugivores, and radiation level were $\log_{10}$-transformed and the local abundance of recently recruited trees and old trees was square-root transformed to normalize data. There was still significant heterogeneity in variances among villages after these transformations, thereby violating assumptions for parametric tests. We assumed that these violations would not have major consequences due to the large number of observations included in each analysis. We explicitly tested for effects of these violations of assumptions by re-doing all analyses using ranked variables. None of the conclusions changed qualitatively following this approach (results not shown). We included all main effects

and two- and three-way interactions in maximal models, eliminating terms using the F test for model selection (Crawley 2002). The final best-fit model was reached when all variables had a significant effect at $P < 0.05$.

We developed best-fit statistical models to assess the relationship between fruit set (response variable) and plant species, radiation, village as a random factor to account for multiple measurements within villages, and plant height (because large plants are expected to produce more fruit, and size may be affected by radiation) and their interactions, as implemented in the statistical software JMP (SAS Institute 2012). These models were subsequently modified by inclusion of abundance of bumblebees and butterflies as two additional predictor variables in order to test for an effect of pollinators on fruit set. Although the abundance of pollinators varies among years, Møller and Mousseau (2011) have shown consistent negative relationships between abundance of butterflies and bumblebees, respectively, and level of background radiation.

At the next trophic level in the food chain, we tested whether the abundance of frugivorous birds could be predicted by abundance of fruit, level of radiation, village as a random factor to account for multiple measurements within villages, environmental variables that could affect the abundance of birds such as time of the day, and weather variables and their interactions.

We developed statistical models for recruitment rate (the local abundance of recruits at a given census point) by relating this to the abundance of older trees, radiation level, and village as a random factor to account for multiple measurements within villages and their interaction. In a second model, we also included current fruit set as an additional predictor variable, and its interactions with abundance of older trees and radiation level to test for effects of fruit set on recruitment rate. This set of models assumes that the relative level of current fruit set of individual trees is representative in terms of relative abundance for fruit set in recent years. Although untested, it seems likely that this assumption is fulfilled because different sites have trees of approximately the same height, the same soil, the same radiation level, and the same pollinator community in different years.

In a final series of analyses, we used means per village to test if the effects found at the level of fruit tree individuals or census points could be extended to the large-scale level of villages.

We estimated effect sizes as a reflection of the strength of relationships, using Pearson product-moment correlation coefficients as our metric. We used the guideline by Cohen (1988) that correlation coefficients of 0.10, 0.30, and 0.50, respectively, explaining 1, 9, and 25 % of the variance, are weak, intermediate, and strong effects.

Results

Abundance of pollinators in relation to radiation

We recorded 345 bumblebees and 439 butterflies during the 898 point counts, mean 0.38 bumblebees (SE = 0.03) and 0.49 butterflies (SE = 0.03) per census point. The most common species of bumblebees were *Bombus terrestris* (74.3 %), *B. agrorum* (13.7 %), and *B. lapidarius* (6.9 %). The most common species of butterflies were *Aporia crataegi* (36.6 %), *Pararge aegeria* (13.8 %), and *Coenonympha pamphilus* (11.0 %). The abundance of bumblebees and butterflies decreased with increasing level of background radiation [Fig. 2a; bumblebees: $F = 3.61$, $df = 15{,}868$, $P < 0.0001$, slope (SE) $= -0.057$ (0.006); Fig. 2b; butterflies: $F = 3.52$, $df = 15{,}868$, $P < 0.0001$, slope (SE) $= -0.027$ (0.007)]. The decrease in abundance across the more than four orders of magnitude range in background radiation levels amounted to 72 % for bumblebees and 40 % for butterflies, leaving the most contaminated areas with few insect pollinators.

Abundance of fruit in relation to radiation and pollinators

We recorded fruit set by 1,329 bushes and trees during the study. The number of apple trees per observation point ranged from 0 to 17, mean (SE) = 1.53 (0.12), $n = 374$, while the number of pear trees per observation point ranged from zero to 18, mean (SE) = 1.71 (0.13), $n = 374$. The number of fruits per census point was 107 (SE = 16) for apple, 91 (15) for pear, 10 (2) for rose, 0.9 (0.7) for rowan, 8 (2) for twistingwood and 76 (18) for European cranberry bush. The abundance of fruit per tree decreased with increasing level of background radiation, as shown for apples in Fig. 2c.

First, we analyzed abundance of fruit in relation to plant species, radiation, plant height, site, and their interactions (Table 1). Fruit set was explained by three predictor variables and three two-way interactions in a model that accounted for 42 % of the variance, while the three-way interaction was not included (Table 1). The abundance of fruit per individual plant varied among plant species, and, unsurprisingly, larger plants produced more fruit (Table 1). The effect of radiation on fruit set varied significantly among plant species as shown by the interaction between species and radiation (Table 1), showing that species differed in susceptibility to effects of radiation. There was a weak effect of species by height interaction, implying that fruit set depended on height in different ways among species (Table 1). Finally, there was a significant interaction between radiation and height, implying that radiation reduced fruit set for a given height (Table 1).
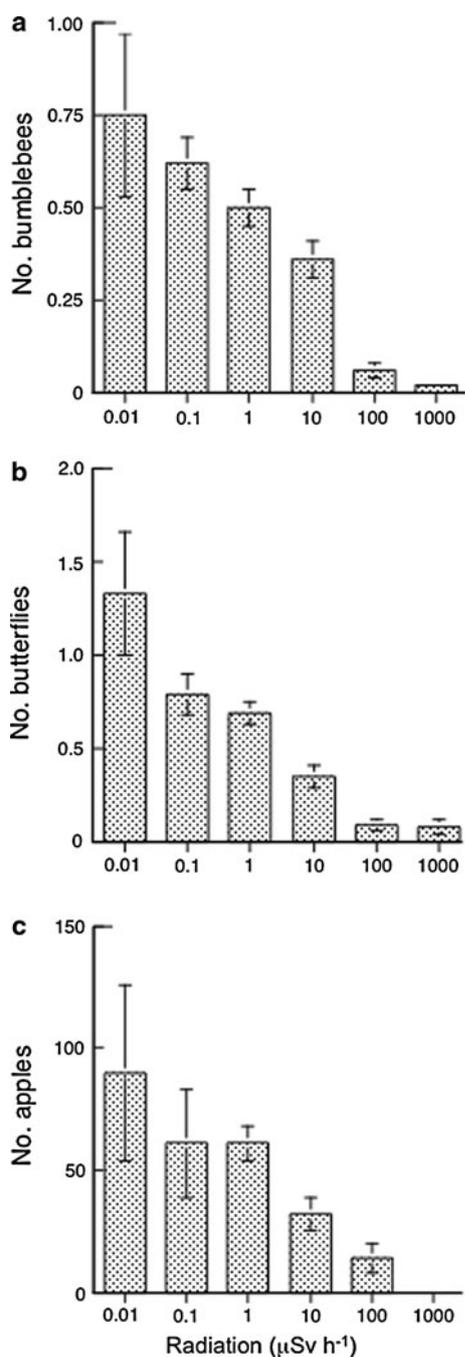
Fig. 2 Mean (±SE) abundance of **a** bumblebees and **b** butterflies per census point, and **c** mean (SE) number of apples per tree in relation to background level of radiation (μSv h^{-1}) around Chernobyl NPP

In a subsequent model we added the abundance of bumblebees and butterflies to the model in Table 1 to test if the abundance of pollinating insects explained variation in fruit set (Table 2). In this model, fruit set was explained by four predictor variables and three two-way interactions that accounted for 27 % of the variance, while the remaining interactions did not reach statistical significance (Table 2). Radiation was significantly negatively related fruit set, with an intermediate effect size (Table 2). In addition, there was a significant positive effect of abundance of butterflies (Fig. 3), with a small effect size, while the effect of bumblebees on fruit set was small and non-significant (Table 2). Unsurprisingly, fruit set varied among plant species, and the effect of radiation on fruit set varied among plant species (Table 2), as shown by the species by radiation interaction. There was a significant interaction between radiation and abundance of butterflies on fruit set (Table 2). The negative sign of this interaction implies that for a given abundance of butterflies fruit set decreased with increasing level of radiation. Finally, there was a significant interaction between abundance of butterflies and bumblebees, suggesting that fruit set for a given abundance of butterflies increased even more when bumblebees were abundant (Table 2). In the remainder of the paper we analyze apples and pears in more detail.

Abundance of frugivorous birds in relation to abundance of fruit and radiation

We recorded between 0 and 34 frugivorous birds at each of the 374 census points, mean (SE) = 0.93 (0.13). The most abundant species was the blackbird with 117 individuals, followed by fieldfare (114 individuals), song thrush (51 individuals), robin (37 individuals), black redstart (12 individuals), blackcap (6 individuals), mistle thrush (5 individuals), and redstart, bullfinch, greenfinch, and garden warbler, each accounting for a single individual.

The abundance of frugivores was explained by a model that included only four predictor variables, accounting for 6 % of the variance (Table 3). The abundance of frugivores increased significantly with local abundance of apples, with a small effect size (Fig. 4; Table 3). Furthermore, the abundance of frugivores decreased weakly with radiation level, and there were small but significant effects of time of day and cloud cover on recorded abundance of frugivores (Table 3). No interaction effect reached statistical significance.

Recruitment by fruit-producing bushes and trees in relation to radiation and abundance of fruit

We recorded 100 recent recruits and 322 old apple trees, giving rise to a ratio of 0.31 for recruits relative to recruiting trees, while for pears the comparable numbers were 196, 305 and 0.64. Plant individuals considered recent recruits were 3.8 m tall (SE = 0.1, n = 100) for apples, while old apple trees were 9.3 m tall (SE = 0.2, n = 322). Similar numbers for pears were for recent recruits 3.1 m (SE = 0.1, n = 196) for recent recruits, while old pear trees were on average 10.7 m tall (SE = 0.3, n = 305).

The best-fit models relating number of recruits at each plot to the number of trees, radiation and their interaction

Table 1 Relationship between fruit set and plant species, radiation level, height, and their interactions for 1,329 fruit bushes and trees around Chernobyl

Variable	Sum of squares	df	F	P	Slope (SE)	Effect size
Species (S)	192.04	5	64.18	<0.0001		0.22
Radiation (R)	47.11	1	78.72	<0.0001	−0.463 (0.052)	0.24
Height (H)	94.31	1	157.59	<0.0001	1.510 (0.120)	0.33
S × R	37.69	5	12.60	<0.0001		0.10
S × H	17.81	5	5.95	<0.0001		0.07
R × H	15.74	1	26.30	<0.0001	−0.657 (0.128)	0.14
Error	783.97	1,310				

Effect size is the Pearson product-moment correlation coefficient. The model had the statistics $F = 51.98$, $df = 18$, $1,310$, $r^2 = 0.42$, $P < 0.0001$. The three-way interaction did not reach statistical significance

Table 2 Relationship between fruit set and plant species, radiation level, abundance of pollinators and their interactions for 1,329 fruit bushes and trees around Chernobyl

Variable	Sum of squares	df	F	P	Slope (SE)	Effect size
Species (S)	127.97	5	34.41	<0.0001		0.16
Radiation (R)	55.15	1	74.15	<0.0001	−0.447 (0.052)	0.23
Butterflies (L)	19.52	1	26.24	<0.0001	0.613 (0.120)	0.14
Bumblebees (B)	1.36	1	1.83	0.18	0.188 (0.139)	0.04
S × R	14.23	5	3.83	0.0019		0.05
R × L	12.39	1	16.65	<0.0001	−0.693 (0.170)	0.11
L × B	8.74	1	11.75	0.0006	1.762 (0.514)	0.09
Error	975.83	1,312				

Effect size is the Pearson product-moment correlation coefficient. The model had the statistics $F = 32.97$, $df = 15$, $1,312$, $r^2 = 0.27$, $P < 0.0001$. The three-way interactions did not reach statistical significance
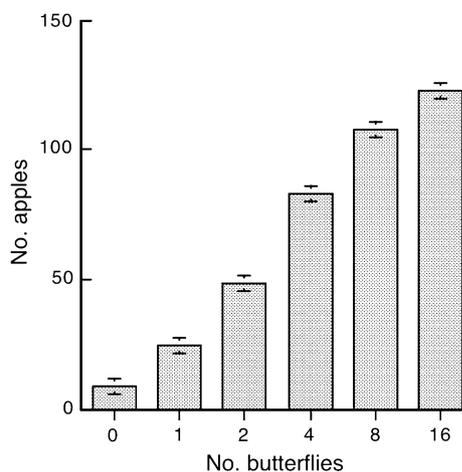
Fig. 3 Number of apples per tree in relation to abundance of butterflies per census point in areas around Chernobyl NPP

included the number of trees, radiationn and number of trees by radiation interaction for both tree species (Table 4). For applesn the relationship accounted for 42 % of the variance in recruitment, while for pearsn this was 51 %. Unsurprisingly, the number of recruits increased strongly with the number of trees for both species,

accounting for large effect sizes (Table 4). There was a significant negative relationship between recruitment rate and radiation (Fig. 5; Table 4). Finally, the significant negative interaction between number of trees and radiation implied that for a given number of trees recruitment decreased even more than linearly with increasing levels of radiation (Table 4).

In a final model, we also included current fruit set as an additional predictor to those listed for the model in Table 4, under the assumption that relative local fruit set is repeatable among years and hence should correlate positively with recruitment rate. The two models explained a similar amount of variance with the same predictor variables having similar effect sizes as the previous models (Table 5). In addition, fruit set also entered with a weak but significant effect size, showing that local recruitment indeed increased with increasing fruit set (Table 5).

Large-scale effects

The abundance of both butterflies and bumblebees decreased with increasing level of radiation among the 19 villages [butterflies: $F = 10.89$, $df = 1,17$, $P = 0.004$, slope (SE) $= −0.139$ (0.042); bumblebees: $F = 8.51$,

Table 3 Relationship between abundance of frugivorous birds and fruit set, radiation level, environmental variables including weather and time of day around Chernobyl

Variable	Sum of squares	df	F	P	Slope (SE)	Effect size
No. apples	0.27	1	4.28	0.039	0.027 (0.013)	0.11
Radiation	0.27	1	4.39	0.037	−0.040 (0.019)	0.11
Time	0.61	1	9.84	0.0018	−0.016 (0.005)	0.16
Cloud cover	0.43	1	6.94	0.0088	−0.088 (0.033)	0.13
Error	22.87	374				

Effect size is the Pearson product-moment correlation coefficient. The model had the statistics $F = 5.84$, $df = 4, 369$, $r^2 = 0.06$, $P < 0.0001$
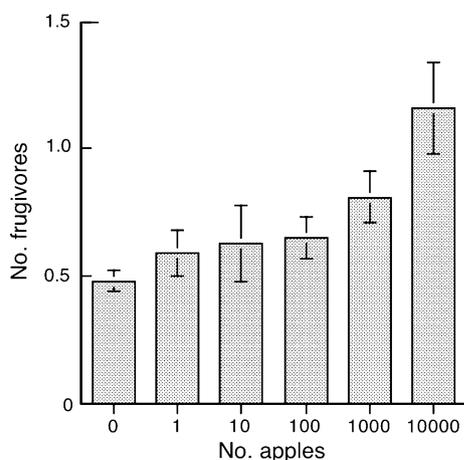
Fig. 4 The abundance of frugivorous birds per census point in relation to abundance of apples per tree in areas around Chernobyl NPP

$df = 1,17$, $P = 0.010$, slope (SE) $= -0.100$ (0.034)]. The amount of fruit per tree decreased with increasing level of radiation across the 19 villages [apples: $F = 18.71$, $df = 1,17$, $P = 0.0005$, slope (SE) $= -0.783$ (0.181); pears: $F = 10.63$, $df = 1,17$, $P = 0.0046$, slope (SE) $= -0.475$ (0.146)]. The amount of fruit per tree increased with the abundance of butterflies [butterflies: $F = 8.44$, $df = 1,17$, $P = 0.010$, slope (SE) $= 2.808$ (0.967); bumblebees: $F = 9.11$, $df = 1,17$, $P = 0.008$, slope (SE) $= 2.040$ (0.676)], while there were no significant effects for bumblebees. The abundance of frugivores increased with the abundance of apples [$F = 8.66$, $df = 1,17$, $P = 0.009$, slope (SE) $= 0.136$ (0.046)], while there were no significant effects for pears or radiation. Finally, the abundance of recruits increased with the abundance of fruit [apples: $F = 10.12$, $df = 1,17$, $P = 0.006$, slope (SE) $= 0.298$ (0.094); pears: $F = 17.60$, $df = 1,17$, $P = 0.0006$, slope (SE) $= 0.500$ (0.119)] independent of radiation. There was an independent positive association between number of recruits and the abundance of frugivores for pear [$F = 6.62$, $df = 1,16$, $P = 0.021$, slope (SE) $= 0.857$ (0.333)].

Discussion

We found a severe reduction in the abundance of pollinating insects in the most contaminated areas around Chernobyl compared to nearby uncontaminated areas in the same neighborhood (Fig. 2). This reduced abundance of pollinators was negatively related to fruit production by bushes and trees. The abundance of frugivorous birds

Table 4 Relationship between number of recently recruited trees and number of trees, radiation level, and their interaction; effect size is the Pearson product-moment correlation coefficient

Variable	Sum of squares	df	F	P	Slope (SE)	Effect size
Apples						
No. trees (T)	38.89	1	142.58	<0.0001	0.438 (0.037)	0.53
Radiation (R)	12.62	1	46.26	<0.0001	−0.279 (0.041)	0.33
T × R	18.68	1	68.46	<0.0001	−0.509 (0.061)	0.40
Error	100.93	370				
Pears						
No. trees (T)	67.16	1	243.66	<0.0001	0.586 (0.038)	0.63
Radiation (R)	19.65	1	71.28	<0.0001	−0.336 (0.040)	0.40
T × R	26.86	1	97.45	<0.0001	−0.549 (0.056)	0.46
Error	101.99	370				

The models had the statistics $F = 90.22$, $df = 3, 370$, $r^2 = 0.42$, $P < 0.0001$ and $F = 126.04$, $df = 3, 370$, $r^2 = 0.51$, $P < 0.0001$ for apple trees and pear trees, respectively
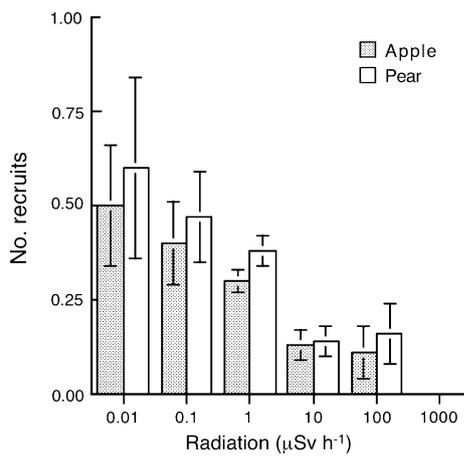
Fig. 5 Mean (SE) recruitment rate of **a** apples and **b** pears in relation to background level of radiation (μSv h^{-1}) around Chernobyl NPP. Values are the number of recent recruits per tree. The value at 100–1,000 μSv h^{-1} is zero

decreased with decreasing abundance of fruit. Finally, recruitment by fruit-producing trees decreased with increasing level of radiation, possibly mediated by a reduction in the abundance of pollinating insects and the amount of fruit. These effects at a local scale were replicated at the large-scale level among villages.

Before discussing our findings in detail, we must emphasize that this study is based on correlational evidence that does not necessarily imply causal relationships. It is also important to note that the effect sizes that we report typically account for 1–10 % of the variance, which is common for ecological studies in any research domain (Møller and Jennions 2002). Pollinators are important bioindicators of the state of the environment including levels

of heavy metals and other pollutants (Kevan 1999). Radiation effects on the abundance of pollinators at Chernobyl were indicated by negative relationships between counts of bumblebees and butterflies and local levels of background radiation, with small to intermediate effect sizes. There are at least two potential underlying mechanisms; direct and indirect effects of radiation on abundance of pollinators. Most radiation around Chernobyl is currently in the topmost 10 cm layer of the soil (Shestopalov 1996; European Union 1998; Yablokov et al. 2009), where most invertebrates live some or most of their annual cycle. Most butterflies lay their eggs in herbs just above the soil, where their larvae subsequently feed. Many larvae pupate in the soil. Adult butterflies spend significant amounts of time in the herb layer. For bumblebees, nests are usually located in the soil or the herb layer, where eggs, larvae, and pupae are exposed to radiation from the soil. Adult bumblebees mainly feed in the herb layer and spend the remaining time in their nests. Thus, there are ample opportunities for exposure to soil layer radiation for these two major taxa of pollinating insects. Therefore, direct effects of radiation on the abundance of bumblebees and butterflies cannot be ruled out. It is also fully possible that pollinators are affected indirectly through contamination of nectar and pollen by radionuclides, as reported for honeybees in Germany exposed to the effects of the Chernobyl disaster (Bunzl et al. 1988). Bumblebees directly feed pollen and nectar to their larvae that then may experience increased doses with consequences for mutations and subsequent viability of their descendants. Likewise, butterflies that typically feed on nectar and pollen may in this way become contaminated and transfer radionuclides to their eggs that then suffer reduced viability.

Table 5 Relationship between number of recently recruited trees and number of trees, radiation level, number of fruits and their interaction

Variable	Sum of squares	df	F	P	Slope (SE)	Effect size
Apples						
No. trees (T)	9.35	1	34.74	<0.0001	0.332 (0.056)	0.29
Radiation (R)	10.24	1	38.04	<0.0001	−0.257 (0.042)	0.31
No. fruits	1.65	1	6.13	0.014	0.102 (0.041)	0.13
T × R	15.47	1	57.50	<0.0001	−0.475 (0.063)	0.37
Error	99.28	369				
Pears						
No. trees (T)	38.00	1	139.34	<0.0001	0.530 (0.045)	0.52
Radiation (R)	18.05	1	66.19	<0.0001	−0.325 (0.040)	0.39
No. fruits	1.36	1	5.00	0.026	0.073 (0.033)	0.12
T × R	24.11	1	88.43	<0.0001	−0.528 (0.056)	0.44
Error	100.62	369				

The other two-way interactions and the three-way interaction did not reach statistical significance. Effect size is the Pearson product-moment correlation coefficient. The models had the statistics $F = 70.14$, $df = 4, 369$, $r^2 = 0.43$, $P < 0.0001$ and $F = 96.80$, $df = 4, 369$, $r^2 = 0.51$, $P < 0.0001$ for apple trees and pear trees, respectively

Fruit set increased significantly with the abundance of butterflies, with a small effect size, while surprisingly there was no significant relationship for bumblebees. Even after controlling for abundance of pollinators, there was still a negative relationship between radiation and fruit set, with a small effect size. We suggest that this apparent direct effect of radiation is caused by elevated pollen abnormality (Kordium and Sidorenko 1997) giving rise to embryo abnormalities and endosperm abortion, as already shown in radiation experiments on domestic apples (*Malus domestica*; Nicoll et al. 1987). However, the relationship between pollen viability and radiation is not consistently negative, with some species showing no relationship and others showing strong negative effects (Kordium and Sidorenko 1997). Two statistically significant interactions predicting fruit set are well worth discussing. First, we found that the relationship between radiation and fruit set differed significantly among plant species. Such interspecific variation in susceptibility to radiation has already been reported for birds which have been found to vary considerably in the generally negative relationship between abundance and radiation (Møller and Mousseau 2007b). Similarly, the frequency of pollen abnormalities can be related to radiation exposure, and also vary significantly among plant species (Kordium and Sidorenko 1997). Second, for a given abundance of butterflies, fruit set was disproportionately reduced at high levels of radiation. This finding implies that an absence of pollinators and radiation act in a multiplicative way to reduce fruit set, the two factors in combination being worse that either on their own. There are no previous studies assessing whether such effects of radiation are additive or multiplicative. Finally, we cannot exclude the possibility that other mechanisms that have not been studied here could account for the observed patterns. All taxa studied so far at Chernobyl have shown negative relationships between abundance and level of radiation, although to varying degrees (Møller and Mousseau 2011). Therefore, it is unlikely that reduced fruit set and recruitment rate at high radiation sites would be caused by increased abundance of flower and fruit predators and phytophagous pests such as insects compared to low radiation sites. Likewise, it seems unlikely that generalist pollinators may have profited from the reduced abundance of specialist pollinators such as bumblebees and butterflies because all taxa investigated so far have shown negative relationships between abundance and radiation (Møller and Mousseau 2011).

Ten different species of frugivorous birds were recorded during our study, with a very heterogeneous spatial distribution. Local abundance of frugivores increased with the abundance of apples. There was an additional intermediate and negative effect of radiation, implying that the abundance of frugivores decreased with increasing level of background radiation. We have previously shown that thrushes, that typically eat considerable amounts of soil invertebrates, are particularly negatively affected by radiation because most radionuclides are currently located in the top soil layer (Shestopalov 1996; European Union 1998; Yablokov et al. 2009) where their prey species live (Møller and Mousseau 2007a). However, we could only account for 29 % of the variance in local abundance of frugivores, leaving most variance unexplained. Therefore, we must conclude that seed dispersal by frugivores is at most weakly negatively affected by level of radiation, suggesting that relatively few seeds disperse from the most contaminated areas.

Recruitment rate of trees depends on abundance of seeds, the quality of these seeds, their growth conditions, and subsequent survival of saplings. There is hardly any empirical evidence suggesting that pollen limitation has consequences for population dynamics of plants (Ashman et al. 2004; Knight et al. 2005). These negative effects of radiation in conjunction with number of trees could act through reduced pollen viability (Kordium and Sidorenko 1997), reduced fruit set (this study), elevated level of embryo abortion (Nicoll et al. 1987), reduced rates of germination, or elevated risk of mortality during the first years after germination. We could show explicitly for the second of these mechanisms acting through a reduced fruit set that indeed fewer recruits were recorded when local production of fruits was reduced, although this was only a weak effect accounting for slightly more than 1 % of the variance. Despite the weak effect, and the fact that we only estimated fruit set in a single year, we were still able to document such an effect on recruitment rate.

We analyzed relationships at the local level of individual plants or census points and found evidence consistent with the expectation that radiation had negative cascading effects on the ecosystem. We extended these analyses to a large geographical scale by investigating relationships among variables at the scale of mean estimates for the 19 villages within and just outside the Chernobyl Exclusion Zone. Interestingly, local scale patterns at the level of individual plants were replicated at the large geographic scale, implying that the patterns reported here may extend to the thousands of square kilometers contaminated with radioactive material in the surroundings of Chernobyl (Fig. 1).

In conclusion, we have shown negative cascading effects of radiation at Chernobyl from pollinating insects over fruit set and frugivory to recruitment of fruit-producing plants. These patterns were extended from local effects at the level of individual plants to the level of villages, showing that radiation from Chernobyl has large-scale effects on ecosystem functioning.

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