

sperm to penetrate the ZP and fuse with the egg plasma membrane [6]. Vaccination with solubilized ZP or isolated ZP proteins can bring about infertility. Sometimes the effects are transient; alternatively, they can be long-lasting, but may be associated with reproductive tissue damage, due to the induction of antibody- and/or T-cell-mediated responses [7].

We cloned rat IE3, a monoclonal antibody that binds the amino terminus of mouse ZP2, a critical ligand for taxon-specific sperm binding [8] (Figure S2A). IE3 transiently inhibits female mouse fertility when introduced as ascites fluid through intraperitoneal injection [9]. Recombinant IE3 localized to the egg ZP *in vitro* and *in vivo* (Figure S2B). When mating was initiated 5 weeks after injection of AAV-IE3, seven out of twelve mice expressing IE3 were infertile, while five were fertile, but with reduced litter sizes compared with controls (mean \pm SEM, 3.8 ± 1.35 vs 14.7 ± 0.94 ; $p < 0.01$) (Figure 1, Figure S2C). However, over subsequent months all AAV-IE3 mice (titer range between 2.8 and 19.3 $\mu\text{g/ml}$) failed to produce progeny, with the exception of one of the initially fertile group of five, who delivered a second litter of one during month three of mating (Figure S2D). Importantly, ovaries from infertile females expressing IE3 for 6 months (Figure 1, compare panels K and J), as with those exposed to IE3 transiently [9], appeared histologically normal, and contained follicles at multiple stages of development, as well as multiple corpora lutea. This, in conjunction with the observation that comparable numbers of eggs can be superovulated from control and AAV-IE3 females (Figure S2B), suggests that vectored expression of anti-ZP antibodies can induce long-term infertility without disrupting normal follicular maturation.

SUPPLEMENTAL INFORMATION

Supplemental information, including background, discussion, hurdles that must be overcome in implementation of vectored contraception, possible strategies for bringing about reversibility, experimental procedures, two figures and one table, can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.08.002>.

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Eurasian reed warblers compensate for virtual magnetic displacement

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Displacement studies have shown that long-distance, night-migrating songbirds are able to perform true navigation from their first spring migration onwards [1,2]. True navigation requires both a map and a compass. Whereas birds are known to have sun, star, and magnetic compasses, the nature of the map cues used has remained highly controversial. There is quite strong experimental evidence for the involvement of olfactory map cues in pigeon and seabird homing [3]. In contrast, the evidence for the use of magnetic map cues has remained weak and very little is known about the map cues used by long-distance migratory songbirds. In earlier experiments [2,4], we have shown that Eurasian reed warblers physically displaced 1,000 km eastward from Rybachy to Zvenigorod (Figure 1) re-orient towards their breeding destinations by changing their orientation in Emlen funnels from the NE to the NW. We have also previously shown that this re-orientation cannot be explained by a ‘jetlag effect’ [5]. We have now used this model system to show that Eurasian reed warblers use geomagnetic map cues to determine their position.

We performed orientation experiments in Emlen funnels with birds captured during spring migration in Rybachy (55° 09' N, 20° 52' E; Figure 1). The birds were housed outdoors in wood and cloth-net cages, which provided the birds with a clear view of the sky and the surroundings (see Figure S1 in the Supplemental Information). First,

we tested the birds' orientation in the natural local geomagnetic field (total intensity 50,650 nT, inclination: 70.3°, declination 5.6°). The birds oriented towards NE (Figure 1C; $\alpha = 25^\circ$, $r = 0.81$, $p < 0.0001$, $n = 17$; 95% confidence interval, CI 7°–43°), which represents the mean orientation of Eurasian reed warblers tested over the previous decade in Rybachy (Figure 1A; $\alpha = 42^\circ$, $r = 0.46$, $p < 0.0001$, $n = 52$; 95% CI 19°–64° [2,4,5]).

Second, we virtually magnetically displaced the birds by continuously keeping and testing them inside the 99% homogeneity volume in the centre of a three-axial Merritt-4-coil system [6] set to generate a magnetic field identical to that found in Zvenigorod near Moscow (55° 42' N, 36° 45' E; total intensity 52,175 nT, inclination: 71.2°, declination 10.1°). For detailed experimental procedures, see Supplemental Information. The Eurasian reed warblers compensated for the virtual magnetic displacement to Zvenigorod by re-orienting their compass orientation towards NW (Figure 1D; $\alpha = 298^\circ$, $r = 0.52$, $p < 0.008$, $n = 17$; 95% CI 264°–332°) just as physically displaced birds do (Figure 1B; $\alpha = 334^\circ$, $r = 0.41$, $p < 0.001$, $n = 52$; 95% CI 308°–360° [2,4]; 95% CI overlap broadly with the virtually displaced birds). The orientation of the Eurasian reed warblers was significantly different before and after the virtual magnetic displacement (Mardia-Watson-Wheeler test: $W = 11.64$, $p = 0.003$, and 99% CIs for the group mean directions did not overlap; 99% CIs 2°–48° in the natural field and 253°–343° after the virtual displacement).

Our data show that a change in magnetic parameters only is sufficient to elicit a re-orientation response towards their breeding destinations indistinguishable from the one seen after a real physical 1,000 km eastward displacement. The birds tested in the present experiments were kept and tested outdoors in cages providing them with free access to all non-magnetic cues including unaltered photoperiod, celestial, olfactory, and landmark cues. Thus, all environmental cues except the magnetic field indicated that the birds were still in Rybachy; only the magnetic field



Figure 1. Results of the virtual magnetic displacement study.

Centre: a map of the capture site (Rybachy, Kaliningrad region), the site of virtual displacement (Zvenigorod, Moscow region) and the breeding range of Eurasian reed warblers in the region (shaded middle grey). The solid arrow on the map shows the virtual displacement direction and distance. The broken arrow at the capture site shows the mean migratory direction of Eurasian Reed Warblers passing through Rybachy, and the broken arrows at the virtual displacement site show our two working hypotheses: (1) no compensation, or (2) compensation towards the breeding destinations (solid line oval, see Supplemental Information for more details). The circular diagrams show the orientation of Eurasian reed warblers tested at the capture site during spring migration 2004–2007 (A) and 2012–2013 (C) and the same birds' orientation after a physical (B) or virtual (D) 1,000 km eastward displacement. The data in circular diagrams (A) and (B) are from [2]. Each dot at the circle periphery indicates the mean orientation of one individual bird; arrows show mean group vectors; the dashed circles indicate the radius of the group mean vector needed for 5% and 1% levels of significance according to the Rayleigh test of uniformity; solid lines flanking mean group vectors give 95% confidence intervals for the group mean directions.

indicated that the birds had been displaced 1,000 km eastward. Our data therefore demonstrate that magnetic cues play an essential role in the map when night-migratory Eurasian reed warblers compensate for a long-distance displacement to an unknown location. The few previous studies investigating the effects of virtual magnetic displacements on songbirds (most recently [7]) reported disorientation rather than re-orientation, which is not very strong evidence for a magnetic map. This disorientation could have occurred for many reasons, for instance because the birds were moved between the altered field and the home field multiple times, which is equivalent to making multiple simulated back-and-forth displacements [7].

The ophthalmic branches of the trigeminal nerves (V1s) are known to transmit non-compass magnetosensory information in a songbird [6,8], and Kishkinev *et al.* [4] previously showed that Eurasian reed warblers need intact V1s to correct for the physical displacements from Rybachy to Zvenigorod. Therefore, the

current study makes it highly likely that at least some of the essential magnetic map information used by Eurasian reed warblers is transmitted to the brain via the ophthalmic branch of the trigeminal nerve. In conclusion, we demonstrate that changes exclusively in magnetic information elicit a compensatory, goalward re-orientation in a long-distance migratory songbird species. Thereby we provide strong evidence in favour of the existence of a geomagnetic map used for long-distance navigation by at least some birds. It might be similar to the magnetic map already known from spiny lobsters [9] and sea turtles [10].

SUPPLEMENTAL INFORMATION

Supplemental Information includes experimental procedures and one figure and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.08.012>.

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Long-term census data reveal abundant wildlife populations at Chernobyl

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Following the 1986 Chernobyl accident, 116,000 people were permanently evacuated from the 4,200 km² Chernobyl exclusion zone [1]. There is continuing scientific and public debate surrounding the fate of wildlife that remained in the abandoned area. Several previous studies of the Chernobyl exclusion zone (e.g. [2,3]) indicated major radiation effects and pronounced reductions in wildlife populations at dose rates well below those thought [4,5] to cause significant impacts. In contrast, our long-term empirical data showed no evidence of a negative influence of radiation on mammal abundance. Relative abundances of elk, roe deer, red deer and wild boar within the Chernobyl exclusion zone are similar to those in four (uncontaminated) nature reserves in the region and wolf abundance is more than 7 times higher. Additionally, our earlier helicopter survey data show rising trends in elk, roe deer and wild boar abundances from one to ten years post-accident. These results demonstrate for the first time that, regardless of potential radiation effects on individual animals, the Chernobyl exclusion zone supports an abundant mammal community after nearly three decades of chronic radiation exposures.

The Belarus sector of the Chernobyl exclusion zone, the Polesye State Radioecological Reserve (PSRER), covers 2,165 km², half of the total area, and has similar radiation levels to the Ukrainian sector (only ca. 1% of the Ukrainian sector is more contaminated). The PSRER provides a unique opportunity to test three key hypotheses concerning the resilience of wildlife to the world's worst nuclear accident.

Hypothesis 1 proposes that mammal abundances are negatively correlated with levels of radioactive contamination at Chernobyl. This hypothesis was not supported by the data. Mean number of tracks per 10 km (2008–2010) was assessed as a function of radiocaesium contamination density on 35 winter survey routes for elk, wolf (Figure 1), wild boar, roe deer, fox, and a combined category of other predatory and non-predatory mammals (see Figure S1 in the Supplemental Information published with this article online). Note that we used radiocaesium contamination density in statistical analyses; radiation dose rates are discussed in Supplemental Information.

For all species, our statistical models (which included habitat variation; see Supplemental Information) rejected radioactive contamination as an important predictor of mammal density within the PSRER. Although census data do not give direct information on population metrics such as reproductive success or longevity, a scenario in which depressed populations in the highly contaminated areas are supported (on a daily basis) by rapid influx and habitat utilization from less contaminated areas seems highly unlikely. Home ranges of the species examined [6] give length scales smaller than, or of the same order as, route length.

A study of small mammals by Baker *et al.* [7] also found no evidence of population declines at Chernobyl. However, a previous study of mammals using track counts [3] reported a negative relationship between radiation levels and mammal density. The discrepancy with our data is likely because this previous study [3] covered only 16.1 km of transects examined just once. Our data are derived from transects with a total length that is 20 times larger and repeated in two (21 routes) or three (14 routes) years.

Hypothesis 2 proposes that densities of large mammals are suppressed at PSRER (Chernobyl zone) compared with those in four uncontaminated nature reserves in Belarus. Again, we found that this hypothesis was not supported by the empirical data. We analysed population density estimates (2005–2010) derived from winter track survey routes and published by the Belarus Ministry of Natural Resources [8]. Similar densities of large ungulates (hoofed mammals) were