Release site manipulation to favour European ground squirrel *Spermophilus citellus* translocations: translocation and habitat manipulation

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Translocating European ground squirrels *Spermophilus citellus* has become a popular conservation tool. However, few release techniques have been carefully evaluated. To contribute to an evidence-based ground squirrel translocation guide for wildlife managers, we evaluated conditions of habitat manipulation (grass height and artificial burrow entrance angle) which we expected to affect settlement of translocated ground squirrels during the critical period after release. In a field experiment, we translocated 173 individuals in southeastern Hungary in 2007. We released the animals into angled or vertical artificial burrows and manipulated grass height. We found that animals preferred angled (~ 30°) artificial burrows, which facilitate digging, and medium-height grass (18 cm ± 1.5). Moreover, although ground squirrels generally are associated with short grass habitats, overhead protection by grasses is valuable after a translocation. This result implies that in order to accomplish a translocation, it is not sufficient to only know the habitat preference of a species in undisturbed situations, but also how and to what extent habitat characteristics should be manipulated to increase the chances of success.

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Translocating animals within their natural distribution range is a popular tool in wildlife management and conservation (Bajomi et al. 2010), and it is often used to reestablish populations of endangered species (IUCN 1998, Fischer & Lindenmayer 2000, Seddon et al. 2007). According to Teixeira et al. (2007), translocations bear three main challenges: 1) survival of the animals after release, 2) settlement of animals in the release area, and 3) successful reproduction. Although there is no general agreement on the criteria for the success of translocations (Seddon 1999), survival in the early post-release period is critical (Griffith et al. 1989, Miller et al. 1999, Gerber et al. 2003, Cheyne 2006, Moorhouse et al. 2009). On the basis of data collected in earlier translocations of European ground squirrels *Spermophilus citellus* (V. Altbäcker, unpubl. data), this early, post-release period is estimated to encompass two weeks post-release for European ground squirrels. During this critical period, mortality was the highest in previous translocations.

Habitat quality at the release site is one of the most important factors to be addressed in a reintroduction (IUCN 1998). For ground dwelling sciurids, the

habitat encompasses both the above-ground and underground environments. For natural colonies of ground squirrels, grass height is one of the most important factors of the above-ground environment to favour survival of colonies (Kis et al. 1998). Animals abandon sites where the grass grows tall and settle where short grassland is established. Ground squirrels have long and elaborated burrow systems (Ružić 1978), which provide a stable and safe environment against predators and unpleasant environmental conditions (Hut & Scharff 1998). Burrow entrances can be divided into two types: vertical burrows (pointing straight down at an angle of 90° to a horizontal surface) and angled burrows (sloping in one direction at an angle of 30-35° to a horizontal surface) (Ružić 1978, Hut & Scharff 1998). The average number of entrances per burrow system and individual is four (Hut & Scharff 1998, V. Altbäcker, unpubl. data).

As ground dwelling sciurids strongly depend on their burrow systems, the use of artificial burrows and acclimation cages is recognised as an essential tool in reintroductions (Anstee & Armstrong 2001, Truett et al. 2001, Simms 2009). During reintroductions of ground squirrels, wildlife managers in Hungary usually apply vertical artificial burrows which are easier to drill. However, there is a lack of data that allow assessing whether angled or vertical artificial burrows better facilitate settlement in the critical period after release.

The European ground squirrel is an obligate hibernator whose reproduction is limited by a relatively short active season, and it is a species of special conservation concern. In the 2011 IUCN Red List of Threatened Species, it is listed as vulnerable with a decreasing population trend (IUCN 2011). However, as the ground squirrel has adapted well to live in grassy recreational parks (Hoffmann et al. 2008) and airports (Váczi et al. 2006), local high densities can create damage and safety concerns, since raptors that prey on ground squirrels may result in collision risks for airplanes, and burrow mounds can be potentially hazardous to small airplanes during landing and take-off. Thus, regulating its abundance by translocations is sometimes a remedy to solve the human-animal conflict (Massei et al. 2010).

In response to the decline in ground squirrel populations, reintroduction as a management tool has been used frequently, with several thousand animals translocated in Hungary and also Poland (IUCN 2010). Nevertheless, only a few projects provide well-documented information that can be linked to the success or failure of these reintroductions. Consequently, nature conservation managers lack an evidence-based translocation guide for ground squirrel translocations.

In our study, we tested important determinants of pre-release habitat manipulation on the reintroduction success. We undertook a combined experiment to test and evaluate how 1) grass height on the release site and 2) entrance angle of artificially drilled burrows can influence successful settlement during the critical period after translocation of wild-caught ground squirrels. Both features can be implemented with careful design during planned translocations without additional costs.

Material and methods

On 14 and 15 July 2007, 173 reproductively active ground squirrels (about 1:1 sex ratio) were captured at Ferihegy International Airport (47°25'8.88"N, 19°15'7.20"E) and were released on 16 July 2007 to Szeri-puszta (46°31'19", 20°04'44"E), a protected grassland (~ 150 ha) in Kiskunság National Park, in the Duna-Tisza Köze region, Hungary. On the release site, ground squirrels went extinct > 10 years before the translocation due to vegetation changes after termination of sheep grazing, and there were no ground squirrels or burrow remnants left. To provide short grass habitat for ground squirrels (Kis et al. 1998), grazing with sheep (Hungarian Merino *Ovis aries aries merino* and Racka *Ovis aries aries racka*) was restarted before our experiment. Additionally, during and after the translocation, we employed predator exclusion in the release site by guarding the animals continuously from hides for two weeks (Rouco et al. 2008). After the animals entered into daily rest in the burrows (including all night), guards applied spotlights for checking the area for predators.

Ground squirrel trapping was carried out by snaring (Gedeon et al. 2011). Until release into artificial burrows, captured animals were housed in separated wire cages ($10 \times 10 \times 40$ cm). All animals were released in the morning of the day after capture, and we used 0.5 litre retention bottles made of glass with low transparency to keep the animals in the burrows. Retention bottles were removed from the burrows the next day when we had confirmed that
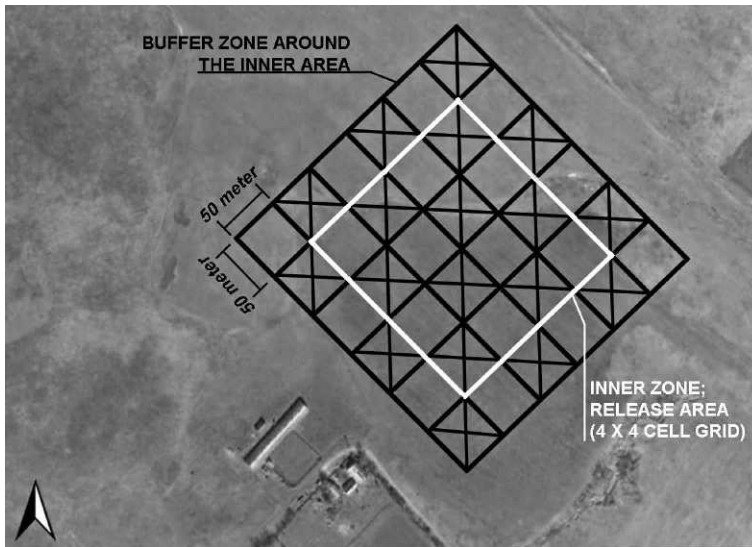
Figure 1. Map of the release site and the experimental design in Szeri-puszta (Google Earth™). Crosses represent grid cells with medium-height grass.

animals were able to dig themselves out from the burrows.

The relocation site (a 6 × 6 cell grid; each grid cell being 50 × 50 m) included a 200 × 200 m inner area (4 × 4 cell grid), where the animals were released, and a 50 × 50 m buffer zone around the inner area (Fig. 1). The release site was signed with signposts (49) in each grid cell corner, and within the inner area (200 × 200 m), 50 cm long and 5 cm wide artificial burrows were drilled with an electric device (Makita BBA520, Japan). We drilled two kinds of artificial burrows: angled and vertical burrows, similar to the natural burrow types (see above). The order of artificial burrow types (angled or vertical) within a grid cell was tessellated and burrows were spaced 12.5 m in all directions. It resulted in a total of 256 artificial burrows in the inner area (16 grid cells), 128 angled and 128 vertical burrows, resulting in equal numbers of burrows in eight grid cells with medium-height (unmown) and in eight grid cells with short (mown) grass (see below). The animals were able to leave the artificial burrow only if they dug themselves out of the burrow as we plugged the animals in the burrow with retention bottles. In order to prevent introducing ground squirrels into an already occupied burrow because previously released animals had moved, all artificial burrows were plugged with bottles before the release. Plugs were removed immediately before the release to that particular burrow and placed back again. This method guaranteed one individual per one artificial burrow.

We released 11 ground squirrels in each grid cell, apart from one grid cell with short grass and two with medium-height grass where we released ten individuals, resulting in a total of 87 and 86 ground squirrels in short and medium-height grass, respectively, at the time of release. As regards the artificial burrows, we released 87 individuals into vertical and 86 individuals into angled burrows. Individuals were assigned to grid cells and burrows arbitrarily and without notice of sex and age. The grass height of the grid cells was alternately mown (short grass; mean = 6 cm ± 0.5; range: 0-14 cm) or unmown (medium-height grass; mean = 18 cm ± 1.5; range: 2-44 cm) by a tractor before the release (see Fig. 1). Grass height was assessed during the experiment at each data record occasion by measuring the height of the longest blade of grass at nine randomly chosen locations within each grid cell (Váczi & Altbäcker 2005).

We noted on a map of the release site if a burrow was occupied by an animal on each data recording day. We defined a burrow as occupied if fresh soil or fresh faecal pellets were at the burrow entrances. After the release, we counted the number of inhabited man-made (angled, vertical) burrows as well as burrows dug by the animals (new burrows) six times in each cell on the release site (1, 2, 3, 7, 37 and 72 days after release; 17, 18, 19, 23 July, 22 August and 26 September). After each data collection, we removed the indicators of occupancy (fresh soil or faecal pellets), so that at the next data collection, we did not record the burrow as occupied based on older signs.

Statistical analysis

Statistical analyses were carried out using the STATISTICA data analysis software system (Stat-Soft Inc. 2009). We adopted a generalised linear modelling (GLM) with repeated measures (time) approach with three categorical variables (grass height, burrow type and burrow form) to predict the number of used burrows. The number of used burrows was counted six times from the time of release of ground squirrels until the end of the growing (active) season. Each categorical variable had two levels (burrow type: vertical or angled; burrow form: new or man-made; grass height: short or medium-height). As the response variable 'number of used burrows' was discrete, we assumed a Poisson distribution (Poisson regression). Because replication of samples was at the grid level (16 grid cells) and each category of burrow type was found only in combination with one of the categories of grass height, we applied a nested design. Because of the nested design, GLM procedures could only use the overparameterised model to compute ANOVAs.

Maintenance of short and medium-height grass during the experiment was tested by a general linear model repeated measures procedure. Between-subject factor was grass height and within-subject factor was time (days after release) with six levels, which corresponded to grass height measurements at six independent occasions.

Summary data are reported as means ± SE, unless stated otherwise. The criterion for statistical significance was always P < 0.05. In figures, we illustrate the changes of grass height or number of inhabited burrows. Values on the x-axis show 'days after release'. As the time interval between consecutive data recording occasions was not even (data recording in days after the release: 1, 2, 3, 7, 37 and 72), it would have been difficult to illustrate the real differences between data recording days. Consequently, we transformed days after release (x-values) by applying a log10 transformation and illustrated these log-transformed values on the x-axis. As a result of this transformation, the difference between x-values does not show the real time difference between days, but it provides better visual illustration of data than an unusually long x-axis with real days from 1 to 72.

Spatial independence of grid cells including used burrows was tested by spatial autocorrelation. A significantly positive coefficient would show spatial aggregation and a significantly negative coefficient would show segregation between data. Since these coefficients compare values for pairs of the average number of used burrows per grid cell, the set of grid cells was divided into distance classes (as lags according to the analysis) of 0, 50, 100, 113, 141, 150, 159, 180 and 211 metres. It means that the distance between the centres of two adjacent grid cells was minimum 50 m and the maximum distance between grid cells was about 211 m. The distance between two grid cells was the linear distance in metres between the centres of the grid cell pairs. According to general recommendations, autocorrelation was tested at lags one to lag $n/4$, where n is the total number of distance classes in the analysis.

Results

Significant grass height difference was maintained between short (6 cm ± 0.5) and medium-height (18 cm ± 1.5) grass grid cells during the whole experiment (grass height: F = 81.64, df = 1, 14, P < 0.001, days after release: F = 11.71, df = 1, 70, P < 0.001, interactions: F = 406.24, df = 1, 14, P < 0.001; Fig. 2). However, plant height on 'medium-height grass' grid cells decreased significantly between 37 and 72 days after release because of extreme drought during the experiment.

The results showed that the number of inhabited burrows changed significantly from the first day after the release until the 72nd day, at which time, all animals disappeared from the surface for hibernation (days after release effect: $\chi^2 = 22.08$, df = 5, P < 0.001; Fig. 3).

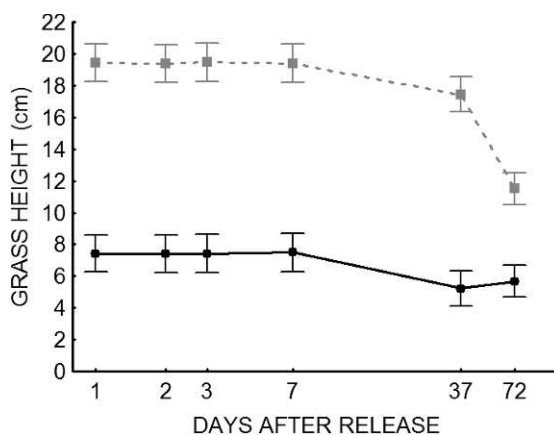The number of inhabited artificial burrows decreased while the number of inhabited new burrows

Figure 2. Change of the 'grass height' in the grid cells of the release site for 'medium-height' (grey) and 'short' grass (black).
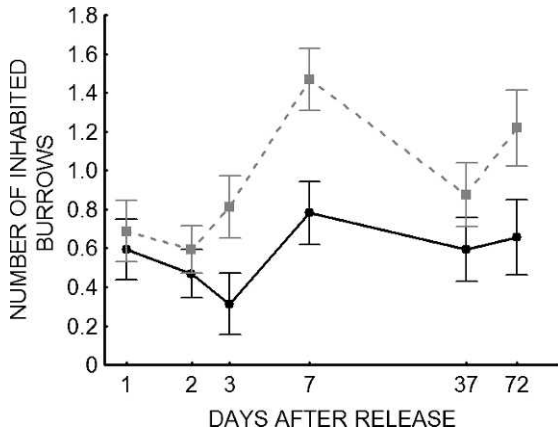
Figure 3. Number of inhabited burrows per grid cell (mean ± SE) in relation to 'days after release' (corresponding to 17, 18, 19, 23 July, 22 August and 26 September, 2007) and 'grass height'. 'Medium-height grass' is shown by a dashed grey line and 'short grass' by a solid black line.

Figure 4. Number of inhabited burrows per grid cell (mean ± SE) in relation to 'days after release' (corresponding to 17, 18, 19, 23 July, 22 August and 26 September, 2007) and 'burrow form (new or artificial)' and 'grass height' (A), and 'burrow type (angled or vertical)' and 'grass height' (B). 'Medium-height grass' is shown by a dashed grey line and 'short grass' by a solid black line.

increased during the experiment (Fig. 4A; burrow form (new or artificial) effect: $\chi^2 = 49.44$, df $= 1$, P < 0.0001, burrow form (new) effect: parameter estimate of 'New' = -0.86, Wald statistic = 45.14, P < 0.0001).

For artificial burrows, the overall difference between occupancy of angled and vertical artificial burrows was not statistically significant (burrow type effect (nested in grass height in the analysis): $\chi^2 = 3.21$, df $= 1$, P $= 0.22$). It also appeared that burrow type effect was not statistically significant for the number of inhabited burrows (burrow type (nested in grass height) effect: P > 0.15). Nevertheless, we found that burrow occupancy changed from the first day until the 72nd day. For only angled burrows, occupancy first increased and then decreased, with a peak on the seventh day after release (see Fig. 4B). Contrarily, the number of inhabited vertical burrows increased steadily from the first day after release.

The χ^2-value for the difference between the number of inhabited artificial burrows in short vs medium-height grass was highly significant ($\chi^2 = 18.06$, df $= 1$, P < 0.0001; see Fig. 3). Thus, burrow occupancy was related to grass height.

Autocorrelation coefficients and figures did not indicate spatial dependencies. In other words, there were no lags with values outside the confidence limit and autocorrelation coefficients were weak and not significant (P > 0.7 for each lags). Consequently, we can conclude that grid cells were spatially independent and there was no sign of serious habitat heterogeneity on the release site.
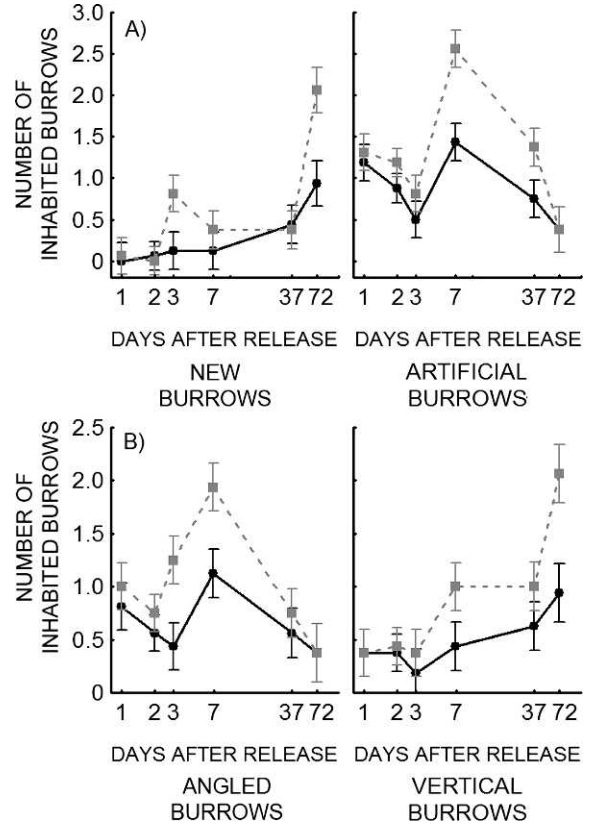
We did not find dead animals in or near the burrows after the translocation (the bottom of artificial burrows appeared empty when investigated using a torch). This shows that plugging with bottles permitted the animals to dig out of the burrows and did not result in extreme cooling or loss of the animals within the burrows.

Discussion

Our results suggest that angled artificial burrows in comparison with vertical artificial burrows and medium-height grass in comparison with short grass favour translocation success of European ground squirrels in the critical period after release. The artificial burrows, as expected from earlier findings (Truett et al. 2001, Simms 2009), provided shelter and refugia for the animals in the critical period

before they excavated their own burrow system. This result is in accordance with the finding of Ružić (1978), whose study implied that angled burrows are dug from the outside in contrast to vertical ones, in which scratch digging is expected to be very hard, because the excavated soil would fall back on the digger.

Environmental conditions (i.e. precipitation and temperature) were stable during the translocation (from release until end of September). It means that random environmental variation did not strongly affect our habitat treatments and their effect on burrow occupancy. Consequently, the end of the critical period can be estimated more closely in the light of the results regarding burrow occupancy (artificial and new burrows). The increasing trend of artificial burrow usage (both vertical and angled burrows) changed to decreasing between the seventh (23 July; highest number of inhabited artificial burrows) and the 37th day (22 August) after the release. In parallel, the number of new burrows dug by the animals began to increase more intensively around the seventh day after release. The opposite trend of the usage of different burrows (artificial vs new and angled vs vertical) in response to days after release may have weakened the specific, individual effect of these parameters in the generalised linear model. Moreover, it seems that grass height effect exceeded burrow type effect, consequently, and because of the nested design, it was not possible to separate these effects. Nevertheless, the results clearly show that angled artificial burrows were preferred over vertical ones in the initial critical period (see Fig. 4B). All in all, we might conclude that the critical period ended between days seven and 37 after the release. This means that within this period, the translocated animals got over the stress caused by capture and release in a new habitat (Teixeira et al. 2007). This finding is in accordance with the result of Calvete & Estrada (2004) who determined the critical period for translocated rabbits *Oryctolagus cuniculus* at about 7-10 days after release. Nevertheless, as we lack additional sampling events during these 30 days, we are not able to specify more precisely the length of the critical post-release period.

It should be noted that numbers of inhabited burrows were always greater than the numbers of observed animals on the release site. This discrepancy requires some explanation. First, microrelief at the release site was unfavourable for visual observations in the area. Second, the size of the animals'

home range (Turrini et al. 2008) and the grid cell area per capita (between 208 and 227 m^2) is comparable in magnitude. Therefore, the animals may have left the home grid cell for feeding and exploration, resulting in positions different from the home grid during the observation periods. Additionally, since surplus (256 burrows were drilled, but only 173 ground squirrels were captured and released), artificial burrows were plugged throughout the study before the animals were released, all burrow occupancies represented one ground squirrel (double counting was avoided by this method; animals were not able to use these extra burrows). Parallel usage of new and artificial burrows cannot be excluded, but one individual usually uses one burrow system (Hut & Scharff 1998, Millesi et al. 1998), and scattered positions of new and artificial burrows implied that parallel use was unlikely. Consequently, we were better able to detect the presence and location of the animals in the grid by counting occupied burrows than by visual scanning or census of animals. Burrow occupancy records seemed a more reliable and conservative technique than visual census.

Our results also suggest that vegetation height at the release site may affect settlement of released ground squirrels. In relocating water voles *Arvicola terrestris*, Moorhouse et al. (2009) concluded that the proper habitat characteristics were vital for long-term persistence. Kis et al. (1998) suggested that ground squirrels prefer short vegetation to tall grass. There are several hypotheses for the preference of short grass in the diurnal ground squirrel under natural conditions: 1) short grass enables predator detection, 2) it helps in communication between conspecifics, and 3) moving and navigation may require less energy in shorter than in taller grass. Newly translocated individuals, however, may have divergent preferences. Our results showed that released ground squirrels at least initially preferred to settle in medium-height over short grass areas, probably because grass tussocks provided overhead protection (see also Ebensperger & Hurtado 2005, Hardman & Moro 2006). Our experience of having difficulties in observing the animals in higher grass grid cells supports this explanation. In general, our findings suggest that it is not enough to know the habitat preference of a species in undisturbed situations, but to learn how and how much we should manipulate the habitat to accomplish a successful translocation action. Experiments such as ours might be helpful.

The success of a translocation should be evaluated

on multiple temporal scales (Seddon 1999, Simms 2009). Post-release monitoring of the released ground squirrels occurred until the end of September 2007, when ground squirrels usually go into hibernation in Eastern Europe (Millesi et al. 1999). In 2008, after the first hibernation, about 30-40 individuals were only observed in an area adjacent to the release site. In 2009, this number increased to 60-80 individuals, occupying a larger area than in the preceding year. Following the generally used visual census technique (Simms 2009) and burrow counting method (Katona et al. 2002, Váczi & Altbäcker 2005, Koshev & Kocheva 2008, our study) to monitor ground dwelling sciurids, the population was estimated at 100-120 individuals (T. Nagy, pers. comm.). The increasing number of animals in these two years seems to fulfil the long-term requirement of a 'successful translocation' (Seddon 1999).

Finally, our experiments support the notion that long-time persistence, which primarily depends on longevity and reproductive success, is strongly connected to the first period after release of the translocated animals, and habitat manipulation at the release site may significantly contribute to successful settlement during this critical period (Letty et al. 2007, Moorhouse et al. 2009).

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