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Spatial behaviour of an overlooked alien squirrel: The case of Siberian chipmunks *Eutamias sibiricus*

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ABSTRACT

Alien species of concern within the European Union have been recently listed and their populations need to be monitored, to plan addressed eradication or control programs. Therefore, the assessment of their presence should be rapidly carried out, particularly for elusive species or for those living at low densities. The Siberian chipmunk *Eutamias sibiricus* is a ground-dwelling squirrel, naturally distributed in northern and eastern Asia. Many introduced populations occur in Europe and Italy too. This species has been listed within the invasive species concern within the European Union and, thus, monitoring is mandatory to manage its potential range expansion. We carried out a hair-tube survey on 31 wood patches in northern and central Italy, where reproductive populations of Siberian chipmunk have been recorded. Hair tubes provided reliable data in assessing the presence of the Siberian chipmunk, with only 1% pseudo-absence and a high detection probability. The occurrence of Siberian chipmunk was positively influenced by study site and by the distance from release site, confirming low dispersal abilities by this species. Dense understorey also affected the presence of chipmunks, preventing them to search for food on the ground and to dig burrows.

1. Introduction

According to a growing number of published studies, biological invasions are the leading cause of the ongoing global biodiversity crisis after habitat loss (Mack et al., 2000; Bax et al., 2003; Clavero et al., 2009). An alien species is defined as invasive if, once introduced outside its native range, it occupies the habitat of native species, altering the structure and the function of native ecosystems and natural environment, by means of hybridization, competition, predation, parasitism and disease spread (Convention on Biological Diversity, Rio de Janeiro, 1992). A species has to pass through three processes to become invasive: introduction, establishment and spread (Lockwood et al., 2009). Therefore, the role of a constant and updated monitoring of the distribution of invasive species is crucial to control their spread and identify areas at risk of invasion. This is in turn necessary to plan addressed management plans to minimize impacts on native biodiversity and environments. In particular, the early detection of new invasions triggers rapid responses, thus strengthening the cost-effectiveness of management programs (Genovesi and Shine, 2004). Detailed knowledge on the distribution of alien species may provide a critical basic information to support prevention of impacts and a strong scientific base for decision-making and resource allocation. This is particularly required for invasive species of concern within the European Union (Carboneras et al., 2017), i.e. those listed within the European Regulation 1143/2014. Currently, suitable methods to monitor low-density or elusive species have been tested on few species (Mills et al., 2000; Thompson, 2004; Kindberg et al., 2009; Srivathsa et al., 2014). When the probability of a direct observation is low or at least unknown, indirect methods, e.g. detection of footprints (Melero et al., 2013; Reynolds et al., 2004; Yarnell et al., 2014), and hair collection through plastic tubes (Gurnell et al., 2004; Harris et al., 2006), represent useful monitoring tools.

The Siberian chipmunk *Eutamias sibiricus* (Laxmann 1769) is a small (95–100 g) ground-dwelling squirrel native to north-eastern Eurasia, from Finland to Korea (Obolenskaya, 2008; Thorington et al., 2012). This rodent is greatly appreciated from the aesthetic point of view, being the most traded squirrel, usually sold as a pet since 1960s (Chapuis, 2005; Marmet et al., 2009). Furthermore, it has been accidentally or intentionally released into forests and urban parks of France, Italy, Belgium, Germany, the Netherlands, Switzerland and

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Japan (Mori et al., 2018). In Italy, populations are recorded in two urban parks in Rome (Villa Ada and Villa Pamphilii), in Sigurtà Garden Park in Valeggio sul Mincio (province of Verona) and along the Piave river in the province of Belluno (Mori et al., 2018). In its native range, the Siberian chipmunk occupies mainly glades of coniferous forests (Obolenskaya, 2008); in Europe, this ground-dwelling squirrel seems to use deciduous forests and fragmented areas with alternating greenwood, woodland and crops (Amori et al., 2009a, b; Marmet et al., 2009; Mori et al., 2018; Thorington et al., 2012). Impacts of the introduced chipmunks are poorly documented in Europe (Mori et al., 2018): competition with small rodents and birds has been hypothesized (Chapuis, 2005) and economical loss has been documented due to damage to cereal and sunflower crops (Freye, 1975). Studies conducted in France have shown that the Siberian chipmunk may contribute to Lyme borreliosis risk more than native rodents, increasing the disease transmission and the risk of human infection (Marsot et al., 2013, but see Vourc'h et al., 2016). A constant monitoring of the distribution of this ground-dwelling squirrel is needed, particularly where the numerical control and eradication of this species are potentially feasible. Differently from tree squirrel species, which are easily detected even through visual sampling (Mori et al., 2016; Chavel et al., 2017), the detectability of Siberian chipmunks may be limited especially where this ground-dwelling squirrel is present at low densities and in areas characterized by a dense understorey. Given that hair tubes have been successfully used for many squirrel surveys (Bertolino et al., 2009; Shuttleworth et al., 2015; Rahim, 2016; Ancillotto et al., 2018), we tested the effectiveness of this method to monitor the presence of the Siberian chipmunk, following Mortelliti and Boitani (2008). The main of this work is to update and clarify the current distribution of the Siberian chipmunk in Italy, by assessing which covariates determine its probability of occupancy and the minimum number of field days required to declare its absence.

2. Materials and methods

2.1. Hair tubes

Hair tubes are PVC segments (length, 25 cm; diameter, ~4.5 cm), baited with hazelnut cream and nuts and horizontally secured on tree trunks. As chipmunks are mainly ground-dwelling squirrels (Thorington et al., 2012), we placed the tubes ~1 m above the ground. Hair-tubes contain two plastic plates, one per entrance, with adhesive placed on the upper side to capture squirrel hairs (Mori et al., 2016). Hair tubes were covered with wood texture paper, to reduce their visibility and to encourage wild rodents to visit them. Wires were used to fix tubes to the trunks.

Hair tubes have been widely used to detect arboreal rodents, even when at low densities (e.g. Eurasian red squirrel *Sciurus vulgaris*: Bertolino et al., 2009; edible dormouse *Glis glis*: Mori and Eusebio Bergò, 2011; hazel dormouse *Muscardinus avellanarius*: Dickman, 1986). Hair tubes have thus been adopted also to early detect new squirrel invasions (Mori et al., 2016), as well as to assess differences in habitat use between native and invasive species (Finnegan et al., 2007). Imperfect detections may occur in hair-tube surveys, thus resulting in pseudo-absences (MacKenzie, 2005). A pseudo-absence is defined as the probability of failure to detect a species when it is present (Gu and Swihart, 2004; Mortelliti and Boitani, 2007; Mortelliti and Boitani, 2008). The problem of false absences is mainly concerning for field studies in biological invasions, as it may substantially affect the effectiveness of management plans (MacKenzie et al., 2002, 2006; Kéry and Schmidt, 2008).

2.2. Study sites and data collection

Occurrences of Siberian chipmunk populations in Italy were obtained to local assessments (cf. Amori et al., 2009a,b; Fiacchini 2010; Mori et al., 2018), integrated with photos sent to a national citizen-science project on squirrels ("SaveRedSquirrel": Mori and Menchetti, 2014). A total of 100 hair tubes were placed in 31 wood patches, located in 8 sites, where the presence of Siberian chipmunk has been reported in the last 10 years (Table 1). Wood patches were separated one-another 250 m. This distance was set according to the spatial behaviour of this species (Marmet et al., 2009; Marmet et al., 2011), to avoid a non-independent sampling, i.e. that the same individual entered in > 1 wood patch. Patches varied in size. Numbers of hair tubes in each wood-patch increased with patch size, from 1 to 5 (Mortelliti and Boitani, 2008); when a patch was smaller than 1 ha, only one tube was positioned. Hair tubes were inspected once a month between July and November 2017, plastic plates changed and bait restored, for a total of 5 checks per site. Logistical constraints imposed a fixed minimum of two visits (Mortelliti and Boitani, 2008).

Table 1
Study sites, number of patches per site and number of hair-tubes per patch.

Study site	Extension	N patches	N tubes/ patch
Sigurtà Garden Park (Verona)	60 ha.	8	1–4
Cesa di Limana (Belluno)	40 ha.	5	3
Valmorel (Belluno)	32 ha.	4	5
Nevegal (Belluno)	16 ha.	2	5
Lentiai (Belluno)	28 ha.	3	4–5
San Silvestro Abate (Fabriano, Ancona)	21 ha.	2	6
Villa Ada – southern part (Rome)	36 ha.	4	1-4
Villa Doria Pamphilii - southern part (Rome)	18 ha.	2	2–3

2.3. Statistical analyses

A binary string of detection history (1, detection; 0, non-detection) was filled for all the visits for each wood patch (MacKenzie et al., 2006). Arboreal rodents recorded within the study areas were: the Siberian chipmunk, the Eurasian red squirrel, the edible dormouse, the black rat *Rattus rattus*, the yellow-necked wood mouse *Apodemus flavicollis* and the striped field mouse *Apodemus agrarius.* Garden dormice *Eliomys quercinus* and hazel dormice were very rare occurrences (Bon, 2017) or absent (Amori et al., 2009b) from study sites. The only species having reddish-brownish, band-coloured hairs longer than 1.5 cm were the Siberian chipmunk and the Eurasian red squirrel (Teerink, 1991; Mori et al., 2016). Hairs were slide-mounted and identified through comparisons with specific atlases and reference collections (Faliu et al., 1980; Teerink, 1991), using an Olympus BX 51 optic microscope equipped with 10-200x lenses. To analyse the cuticular patterns, hairs were mounted on a slide by placing them on a layer of transparent nail polish. After it was completely dry, the hair was removed and observed at the microscope. To analyse the medulla, the hair was longitudinally sectioned and wet with cedar oil at the section level. Hairs of Siberian chipmunks are easily distinguished from those of native red squirrels, as showing a roundish section (which is kidney-shaped in red squirrels), a mosaic-scaled cuticular pattern in the central part of guard hair (regular-waved in red squirrels), multicellular medullar composition (which is thicker in red squirrels) (Faliu et al., 1980; Teerink, 1991).

Analyses were run using the software R 3.3.2 and the package *Unmarked* (Fiske and Chandler, 2011). We selected single seasons models, as our survey was carried out in summer-autumn 2017. We included the effects of the following covariates: [Cov1], study site; [Cov2], average light intensity (as a proxy of the canopy density, measured through the © KHTSXR Luxmeter App for Android smartphones: cf. Negar et al., 2014); [Cov3], Euclidean distance from the release site (we referred to multiple release sites depending on the population considered, detailed in Mori et al., 2018); [Cov4], percentage of understorey cover and [Cov5], patch size. The percentage of

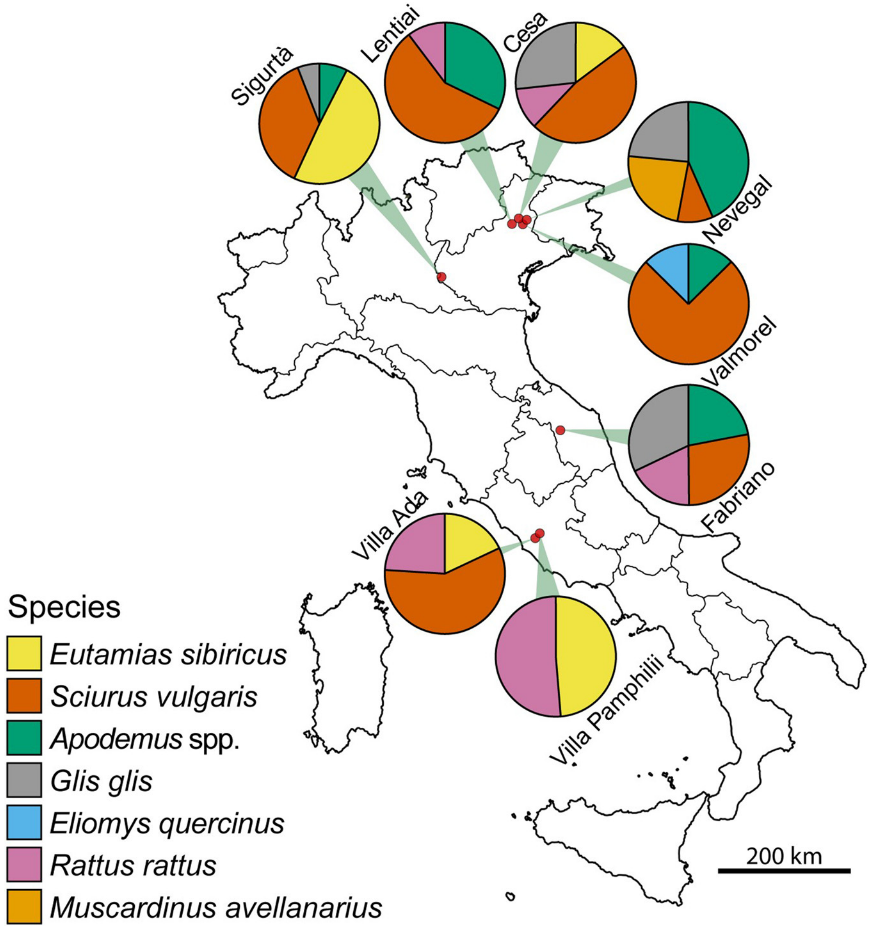

Fig. 1. Percentage of detection of each species through hair tube survey, for each study site.

Table 2
Summary of the parameter estimates for the occupancy. Models are ranked according to the AIC_C, w_i (Akaike weight), -2LL (-2 Log-likelihood), ψ (proportion of sites occupied), p (detection probability), SE (standard error), N (sample size estimate). Cov1, study site; Cov3, distance from the release site; Cov4, percentage of understorey cover; A.P.E., model averaged parameter estimates, Significant ΔAIC_c are those with values < 2 (bold values).

Model	AIC_C	−2LL	ΔAIC_C	w_i	ψ (SE)	p (SE)	N
ψ (Cov1 + Cov3)p(.)	**94.95**	**88.95**	**0.00**	**0.7717**	**0.47 (0.04)**	**0.58 (0.07)**	**3.45**
ψ (Cov1 + Cov3 + Cov4)p(.)	**96.82**	**88.82**	**1.87**	**0.2794**	**0.48 (0.01)**	**0.57 (0.07)**	**3.46**
ψ (Cov1 + Cov3)p(Cov1)	105.48	99.48	10.53	0.0037	0.49 (0.05)	0.59 (0.09)	3.35
ψ (Cov3 + Cov4)p(.)	106.52	100.52	11.57	0.0022	0.38 (0.09)	0.60 (0.08)	3.26
ψ (Cov3)p(Cov1)	108.26	104.26	13.31	0.0009	0.44 (0.06)	0.57 (0.04)	3.54
A.P.E.					0.47	0.58	

understorey cover was measured as a categorical variable with 4 levels following Rhim and Lee (2001): we selected a 5 m diameter circle within each patch and we measured the level of understorey cover within it.

Tubes included within each wood patch were not independent, i.e. we cannot rule out that the same individual chipmunk have visited more than one of them each month (Yarnell et al., 2014). Therefore, we ran our occupancy model on wood patches. We accounted for uncertainty in model selection through a multi-model inference approach and then we estimated the parameters (p - the detection probability - and ψ - the occupancy probability) through model averaging (Burnham and Anderson 2002). Models were fitted following Mortelliti and Boitani (2008): we started with a null model, then we focused on ψ, then on p. We evaluated the sample size, i.e. the minimum number of visits necessary to infer the absence of the Siberian chipmunk, following Reed (1996): $N = \ln(\alpha)/\ln(1\text{-}p)$, where α represents the probability of type I error (i.e. 0.05) and p the detection probability. Models were ranked according to the values of Akaike Information Criterion corrected for small samples (AIC_C) (Burnham and Anderson, 2002). As different models may provide us with similarly significant outputs, we considered as equally valid models which presented a $\Delta AIC_C < 2$ (difference in AIC scores between the best model and the i^{th} model: Burnham and Anderson, 2002). Akaike weights indicate which models were best supported by the data in the model set (Burnham and Anderson, 2002). Wood patches with hair tubes were also traveled by walking during all the visits ($n = 5$), to directly count observed chipmunks. All the direct counts were opportunistically carried out at midday, during the activity peak of this species, and in sunny days to avoid biases due to weather conditions. We tested whether the percentage in hair tubes visited by chipmunks was correlated (Pearson's correlation coefficient) with the number of observed individuals within the same patch.

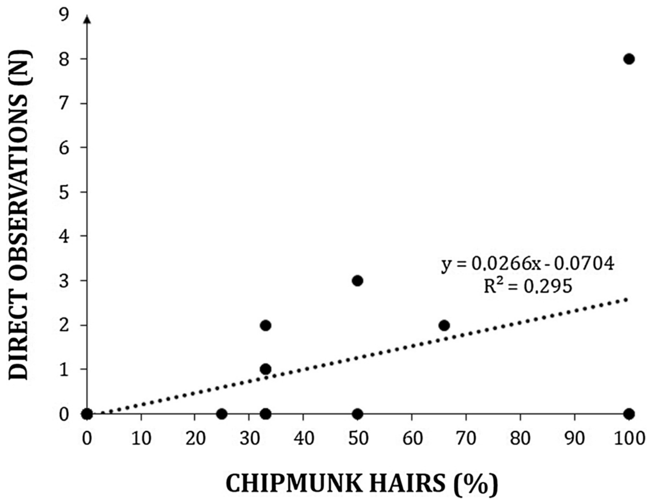

Fig. 2. Relationship between the mean percentage of hair tubes with chipmunk hairs and the number of direct observations. The equation of the trend line and relevant R^2 is reported.

3. Results

Chipmunk hairs were detected in 46 out of 100 hair tubes (Fig. 1). The visit by one species does not prevent the visit by a second species: hairs of > 1 species were recorded in 2% tubes. The percentage of sites in which the Siberian chipmunk was actually found (naïve estimate = 0.46) was only 1% smaller than the estimate of proportion of occupied sites ψ (0.47: Table 1): thus, our sample included 1% of pseudo-absence, i.e. the Siberian chipmunk was also present in 1% sites where it has not been detected.

The best model (Table 2) considers the probability of presence as a function of the interaction of two covariates: the study site and the distance from the release site. This model also showed a high w_i (77%). Despite the second model has a $\Delta AIC_C < 2$, we discarded it, as being very similar to the first one but with a covariate more, thus reducing its parsimony (cf. MacKenzie et al., 2006). The covariate effect was positive for the distance from the release site ($\beta \pm SE = 0.32 \pm 0.09$) and negative for the understorey cover ($\beta \pm SE = -0.13 \pm 0.05$), indicating that patches near the release site were more likely to be occupied by chipmunks, whereas the greater the understorey cover the lower the chance for these patches to be occupied by chipmunks. The detection probability (p) was high for both the selected models ($p = 0.58$). According to our results, four repeated visits are sufficient to detect if the Siberian chipmunk is present in the study area. The percentage of hair tubes with chipmunk hairs and number of individuals observed in 4 visits in each study site were significantly correlated (Pearson's correlation coefficient $R^2 = 0.74$; $P = 0.001591$: Fig. 2).

4. Discussion

Our work provides a reliable summary on the distribution of the Siberian chipmunk in Italy, therefore updating previous reports. In Italy, this small ground squirrel showed the widest population in Sigurtà Garden Park (province of Verona, NE Italy), whereas few individuals are present in Rome (Villa Ada and Villa Doria-Pamphilii urban parks). The population established along the Piave river, which was considered to be the largest one in Italy (Bon 2017) seems to be almost extinct (province of Belluno, NE Italy), with only 1–2 individuals occurring in semi-captivity (i.e. partially fed by humans) in the surroundings of the release site in Cesa di Limana. In the last other sites of the same province (i.e. Valmorel, Nevegal and Lentiai), the Siberian chipmunk was not detected. All the other areas where chipmunks were recorded during the last ten years were surveyed (Fiacchini, 2010; Mori et al., 2018), with the only exception of Dobbiaco (province of Bolzano, NE Italy), where the four individuals set free were detected dead by the owners of the private zoo (Mori et al., 2018). In facts, the detection probability of the Siberian chipmunk using baited hair tubes is quite high. Accordingly, the number of sites where the species was actually present was only 1% lower than the proportion of sites occupied. Four visits, once a month, were estimated to be sufficient to infer the absence of the Siberian chipmunk. This estimation is not absolute, but related to this protocol and can be applied in environments similar to our study areas (Mortelliti and Boitani, 2008). AIC_C values and weights provided a strong evidence for the first model. This models showed that the occurrence of the Siberian chipmunk was influenced by two covariates: the study site and the distance from the release site. The presence of alien chipmunks has been reported to be influenced by the management of the woodland, as they mainly occur in urban/suburban areas (Mori et al., 2018), where tourists and park visitors use to feed them. All the releases in Italy occurred in urban areas, and, given the low dispersal abilities by this species, most populations successfully settled and spread in green areas within cities (e.g. Sigurtà Garden Park: Mori et al., 2018). The inclusion of the percentage of undergrowth cover in the second model reflects the ground-dwelling behaviour of the Siberian chipmunk (Thorington et al., 2012). Where the understorey is too dense, food search by chipmunks on the ground might be prevented, as well as burrow system digging (Jo et al., 2014). By contrast, the number of hair tubes in each wood patch did not affect the detection probability of the rodent. The percentage of hair tubes with chipmunk hairs detected along the control visits to each transect and the mean number of individuals observed during our surveys were correlated. However, no studies have validated yet the direct observation of Siberian chipmunks along linear transects as an accurate system to estimate population size and density. Thus, currently, it is not possible to assess the population density of this ground-dwelling squirrel by inferring it through results of hair tube surveys: different individuals visiting a single tube will count as one individual. This could lead to an underestimation of the real detection probability, but will not affect the inference on the species absence.

Since its introduction outside its native range, the impact of Siberian chipmunk has been overlooked with respect to other invasive squirrels, mostly because of its localized distribution (Bertolino et al., 2000, 2015; Mori et al., 2018). Despite this, given that this squirrel may help the spread of parasites and diseases harmful to humans (Marsot et al., 2013), it has been included within the list of invasive species of concern within the European Union (Carboneras et al., 2017). As to other invasive squirrels, chipmunks are highly appreciated by the general public (Mori et al., 2018). Thus, numerical control and eradication of alien chipmunks are challenging operations and require effective strategies and high effort, as ground-squirrels are highly appreciated by the general public (Bertolino, 2009). Monitoring invasive species of concern within the European Union is recommended, particularly to detect new areas of invasion, as well as to define the current range of introduced squirrels (Genovesi and Shine, 2004; Carboneras et al., 2017). Indirect monitoring method which do not involve direct observations should overcome the problems of low detectability of this species, especially when present at low population density or in areas characterized by a dense undergrowth.

To conclude, hair tube surveys were confirmed to be cost-effective also to monitor alien chipmunks, providing useful results in a low amount of time. Accordingly, this method provided good results, with only 1% of pseudo-absences. In the future, the power of hair tube surveys to evaluate density of chipmunk populations should be tested, given the difficulties to quantify their consistencies through direct surveys.

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