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Original investigation

Pine marten density in lowland riparian woods: A test of the *Random Encounter Model* based on genetic data

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ABSTRACT

In marginal habitats, populations should reach lower densities, as a consequence of both lower overall abundance and increasing home range size. To test if recently colonized riparian woods crossing intensively cultivated lowlands of NW Italy represent marginal habitats for the forest-specialist pine marten *Martes martes*, we assessed its population density by a recently developed, camera-trap-based non-invasive method, the Random Encounter Model (REM). As the central assumption of the REM is that animals move randomly with respect to cameras, we suspected that this method may be unsuitable for species with a strong tendency to use linear elements of the territory as usual paths and select habitats, such as woodland, which are likely to be under-represented in fragmented landscapes. To test for the efficacy of the REM, we also applied a faecal DNA-based genetic census to obtain an independent estimate of the minimum number of individuals occurring in the study area. Camera-trapping used 10 camera-traps, deployed for 10 days within a 2 km^2 large unit, for a total of 6 units and 12 km^2. Pine marten density was estimated at 0.48 (0.36–0.60) ind/km^2. All the faecal samples identified by a mDNA-based PCR-RFLP method as pine marten were genotyped at 15 microsatellite loci using a multiplex protocol. We identified 15 different individuals, corresponding to a density ranging between 0.8 and 2.0 ind/km^2. Using the most conservative genetic estimate, the REM underestimated population density of about 60% proving to be unreliable for estimating pine marten population size. We suggest that this may be the case for elusive species for which the assessment of average daily movements cannot be achieved without the use of invasive methods.

Introduction

Population distributions, range boundaries and their variation over time are regulated by several biotic and abiotic factors, including climate, interspecific interactions, food availability and anthropogenic effects (Case et al., 2005; Holt et al., 2005; Chen et al., 2011). Although the classical biogeographical hypothesis that species' abundance declines monotonically from the centre towards the edges of their range has been recently criticized as being too simplistic (Sagarin et al., 2006), several empirical studies have demonstrated that edge populations differ from those near the core of the range in several demographic characteristics. In marginal habitats, populations reach lower densities (Vucetich and Waite, 2003), as a consequence of both lower overall abundance (Gyllenberg and Hanski, 1992) and increasing home range size (Dunning et al., 1992), tend to produce less offspring (Kawecki, 2004; Thingstad et al., 2006), are more prone to extinction for stochastic causes (Antonovics et al., 2006), and often show lower genetic diversity (Hoffman and Blouin, 2004; Tsumura et al., 2007) compared to core populations.

The pine marten, *Martes martes*, is distributed throughout much of Europe and northern and central Asia, where it is generally associated with mature forest habitats (Proulx et al., 2004; Zalewski and Jedrzejewski, 2006). Recent studies, however, have suggested that the species is more catholic in terms of habitat preferences than previously reported (Virgós et al., 2012), being also able to live in apparently unsuitable rural landscapes that contain remnant forest patches (Pereboom et al., 2008; Balestrieri et al., 2010; Mergey

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et al., 2011; Caryl et al., 2012). Despite the supposed lower suitability of fragmented habitats, Mergey et al. (2011), in France, did not find any clear effect of the degree of habitat fragmentation on either individual space-use patterns (temporal stability and size of home ranges; Mergey et al., 2011) or genetic variability of pine marten populations (Mergey et al., 2012). However, Ruiz-González et al. (2015) found that habitat loss and fragmentation, in synergy with past overharvesting and possible interspecific competition with sympatric stone marten (*Martes foina*), are likely to be the main factors responsible for recurrent bottlenecks and low gene flow in heterogeneous landscapes.

In northern Italy, the pine marten has traditionally been associated with deciduous and coniferous forests ranging in elevation between 1000 and 2000 m a.s.l. (e.g. Prigioni et al., 2001). Since the end of the 20th century, pine marten records in intensively cultivated areas of the western River Po plain (NW Italy) have grown exponentially, suggesting a progressive range expansion throughout the interior of the plain (Balestrieri et al., 2010). Non-invasive genetic monitoring revealed that pine marten occurrence in several residual woodland patches was stable (Remonti et al., 2012) and that riparian woods may play the role of natural corridors favouring the southward expansion of the pine marten (Balestrieri et al., 2015).

Having been colonised only recently and deeply altered by human economic activities, the agricultural habitats of the River Po plain may be expected to represent marginal habitats for the pine marten, acting as dispersal sinks with high turnover of extinction-(re)colonisation events (Kawecki, 1995; Baguette, 2004). To test for this hypothesis, we aimed to assess pine marten density on a stretch of the River Ticino for which the first records of pine marten occurrence dated back to 2005. Like most carnivorous mammals, pine martens are elusive and difficult to monitor (Ruiz-Gonzalez et al., 2008; Rosellini et al., 2008). In the last two decades, camera-trapping has become a widely used, non-invasive method to assess through mark-recapture models the density of species with individually unique coat markings (e.g. Karanth and Nichols, 1998; Maffei et al., 2005). Rowcliffe et al. (2008) have proposed a modified ideal gas model (*Random Encounter Model*; REM) that does not depend on individual recognition and can be potentially applied to a wide variety of species. The model has been developed and tested in southern England for four non-native species, free ranging in a 226 ha large enclosure mostly consisting of open grassland (Rowcliffe et al., 2008) and then has been applied to the pine marten in central Italy, yielding density values consistent with available data for the European marten range (Manzo et al., 2012).

There are three key assumptions for this model, that: (i) animals behave like ideal gas particles, moving randomly and independently of one another, as so as the expected number of photographs depends simply on the ratio of the area covered by each individual to the total area within which they are sampled; (ii) animals move independently of the cameras; and (iii) the population is closed (Rowcliffe et al., 2008). Although the model is considered to be robust to behavioural patterns that may violate the first assumption (Rowcliffe et al., 2008), when density estimates have been compared to those obtained by other methods large discrepancies have been reported (Anile et al., 2014; Cusack et al., 2015; Caravaggi et al., 2016). The central assumption of the REM is that animals move randomly with respect to cameras (Rowcliffe et al., 2013) and thus this method may be unsuitable for species with a strong tendency to use habitats which are under-represented in fragmented landscapes. Pine martens strongly avoid open habitats (Storch et al., 1990; Brainerd and Rolstad, 2002) and move close to tree-cover (Pereboom et al., 2008; Del Fante, 2012), suggesting that the sampling protocol required by the REM may lead to underestimation of pine marten density.

To obtain an independent estimate of pine marten density and test the efficacy of the REM for the assessment of meso-carnivore density in fragmented habitats, in the same area and period monitored by camera-trapping, we applied a non-invasive faecal-DNA based genetic census (Ruiz-González et al., 2013). Genotyping needed two steps, species identification, which was necessary to exclude the samples belonging to other sympatric meso-carnivores from further analyses (Ruiz-Gonzalez et al., 2008), and microsatellite genotyping, to ascertain with >99% certainty the minimum number of individuals occurring in the study area (Ruiz-González et al., 2013).

Material and methods

Study area

The Italian stretch of the River Ticino flows southwards from the southern edge of Lake Maggiore to the median course of the River Po, forming a valley 110 km long and, on average, 7 km wide. The valley is partly protected by two Regional Parks: the Park of the Ticino Valley (Lombardy), covering 906.4 km^2 and the Natural Park of the Ticino Valley (Piedmont), 62.5 km^2. The river crosses an intensively cultivated and urbanized plain, where mesophilous – *Fraxino-carpinion* – and hygrophilous – *Alno-Ulmion*, *Alnion glutinoso-incanae*, *Salicion albae* – woods are still widespread inside the weave of meanders, streams, canals and oxbow lakes which characterise the downstream stretch of the river (Fig. 1). The climate is temperate, mean annual values being 13 °C for air temperature and ca. 700 mm for rainfall.

Pine marten monitoring focused on the lower part of the valley, between the towns of Vigevano and Abbiategrasso (Milan, Lombardy) in the north and Gropello Cairoli village (Pavia, Lombardy) in the south. Along this ca. 35 km long stretch of the river, mean percent riparian vegetation cover, as assessed in a 100 m large belt on both river banks, is 47.8% (min-max: 12–86%; Prigioni and Balestrieri, 2011). Long-term non-invasive monitoring showed that currently stone marten occurrence in this sector of valley is negligible (Balestrieri et al., 2010, 2015).

Estimation of population size by camera-trapping

The study area was monitored by digital scouting pocket cameras (two Multipir 12 and eight SG550 with Passive Infra-Red motion sensor), provided with 8 GB SD cards and up to 8 AA batteries. Cameras were tied to Trees 30–50 cm above the ground level. In accordance with Rowcliffe et al. (2008), they were neither baited nor set at sites thought to have high animal traffic (trails or obligated passing places). Cameras were set to record 15 s long videoclips, with a 30 s interval between two successive recordings. Videoclips allow a more precise identification of the species recorded than pictures, while the delay period was set as to provide a balance between continuous monitoring and memory wastage through multiple recordings of a same animal.

There are tradeoffs in the setting of camera-traps because too close trap-spacing may reduce the number of individual home ranges surveyed whereas too wide trap-spacing may result in the missing of those individuals whose home ranges are included in the "holes" between camera-traps (Kays and Slauson, 2008). Since in Italy, pine marten home range has been reported to vary between 160 and 548 ha (mean: 370 ha, N = 6; Antonelli, 1996; Del Fante, 2012), surveys were carried out using a 1 × 1 km grid, superimposed on the kilometric grid of digitalized, 1:10,000 Regional Technical Maps; for each session, 10 camera-traps were deployed for 10 days within a 2 km^2 large unit, for a total of 6 units, 12 km^2 (Fig. 1) and, potentially, 600 camera-days. Deployment time coincided with