# **Multi-scale assessment of ecological corridors core-matrix-barrier systems for wildlife populations connectivity**

### INTRODUCTION

Landscape structure affects intraspecific connectivity among populations (Kubisch et al. 2014). This connectivity, however, is challenged by fragmented landscapes, negatively affecting gene flow between populations and consequently their long-term viability. In a patchy landscape, populations tend to be more sensitive to disturbances and are further susceptible to extinction (Newman and Pilson 1997, Loeuille and Leibold 2014). Remnant small, isolated populations, may undergo processes of declining gene diversity by genetic drift and/or inbreeding depression. Connectivity among sites can determine whether the species will fall into a small fragmented population or will develop a meta-population structure (Hanski 1999). At the molecular level, if a certain species is limited to particular landscape elements and gene flow among suitable elements is prevented, populations are expected to show a decrease in their genetic variability, followed by an increase of their sensitivity to harsh stochastic events (Nevo et al., 1984; Primack, 2000). Accordingly, the loss of genetic diversity within a patch will be a function of the degree of isolation of the sub-population, which will be countered by mutation and dispersal among the sub-populations. In this situation, species are also expected to show elevated genetic differentiation among populations (Nahum et al., 2008; Avrani et al., 2012; Peled et al., 2014, 2016).

The concept of biological connectivity via ecological corridors (ECs) is based on the use of open landscapes that are not necessarily of high ecological value, but presumably still enabling a certain degree of organisms exchange and gene flow between populations inhabiting spatially separated natural habitats (Wilson and Willis 1975, Jongman and Pungetti 2004, Gilbert-norton et al. 2010, Marrotte et al. 2017, Hilty et al. 2019). This concept, raised by academic research, is now recognized as a practice in the frontline of nature conservation (Eldredge 2000, Anderson and Jenkins 2005, Correa Ayram et al. 2016, Hilty et al. 2019). One of the main forces behind this process is the rapid human population and economic growths, which drives expeditious anthropogenic development that results in significant natural land loss and ecosystems fragmentations (IPCC 2019, Sorek et al*.* 2019). These extensive changes in land use are recognized as one of the most significant factors affecting what is now widely accepted as a current biodiversity crisis (Eldredge 2000, Singh 2002, Haddad et al. 2015, Tilman et al. 2017, Driscoll et al. 2018) and have been shown to be a strong determinant of species distributions at multiple spatial scales from local roadside (Erinjery et al. 2017) to regional, national and continental scales (Kent et al. 2011, 2014), causing potential adverse effects on ecosystem continuity (e.g., tropical forest canopy continuity, (Kent et al. 2015, Erinjery et al. 2018)) which may affect all life stages and mobility of local species (Erinjery et al. 2019, Erinjery et al. In review, Sorek et al. 2020).

Since the consciousness rise of the subject by Shkedy and Sadot (2000), practically all conservation entities in Israel, governmental or NGOs, have put forward and addressed the subject (Rotem et al. 2015, Ministry of Environmental Protection 2017; Gabay and Zanzuri 2019; Sorek et al. 2019; Sorek et al. 2020). Furthermore, following the 2016 insertion of a national ecological corridors scheme into the Israeli National Outline Plan (Tama 1, 2020), regional schemes proposing to establish a statutory status for the protection of ECs are currently in development (Ministry of Environmental Protection 2017, Shapiraet al. 2020).

There is however, a substantial lack of knowledge regarding the efficiency of corridors and passages in maintaining terrestrial habitat connectivity among target species populations (Kindlmann and Burel 2008, Gilbert-Norton et al. 2010, Closset-Kopp et al. 2016, Shapiraet al. 2020). Moreover, it is highly probable that organism requirements and ECs functionalities are highly specific not only for the organism, but for the interaction between a species and the EC's local characteristics and conditions (Hess and Fischer 2001, Driscoll et al. 2013, LaPoint et al. 2013, Scharf et al. 2018), stressing further the need for specific, local knowledge of ECs functionality. This leads to one of the main dilemmas in practicing nature conservation in general, which is the need to balance between the need for immediate action without sufficient empiric scientific data. This phenomenon is generally known as the scientific-practice gap (Bertuol-Garcia et al. 2018). This gap is very apparent in the planning of ECs in Israel, as the need to protect open landscapes is pressing, but there is a substantial lack of empiric data regarding ECs functionality in maintaining terrestrial habitat connectivity. When designing an EC, the process should, ideally, include specifying the EC's goals, understanding the biological and ecological requirements of the organisms that the EC is supposed to serve and to assess the EC's ability to provide these requirements (Hess and Fischer 2001, LaPoint et al. 2013, Scharf et al. 2018). Obviously in many cases, conservation actions are required even if data is scarce or absent (see for example Shamoon et al*.* 2018, Shapira et al. 2019). Ideally, however, acquiring data should be a continuous goal regardless of the timing of the action at hand, for both future improvements of the area itself and further decision making.

In the current proposal, we aim to examine the efficiency of Israel’s CMB systems in maintaining connectivity between populations of wildlife species existing in natural habitat patches. For this purpose, we suggest using a combined approach, including spatial analyses, wildlife activity monitoring and genetic analyses of populations of target species in natural habitats along the corridors to estimate gene flow barriers and the isolation levels among populations. This goal will be achieved by comparing wildlife communities and populations, including genetic variabilities, between natural habitats with different degrees of spatial and temporal separation.

We propose to assess ECs and passages efficiency, and to evaluate the implications of compromising the integrity of ECs put forward by the Israel Parks and Nature Authority (INPA). As defined by the INPA, the majority of the ECs in Israel consist of a mosaic of natural, semi-natural and agricultural land uses, including narrow bottlenecks (Shkedy and Sadot 2000, Rotem et al. 2015, Gabay and Zanzuri 2019, Shapira et al. 2020). This system imposes variable conditions, leading to heterogeneous and complex matrix systems we define hereafter as a core-matrix-barrier (CMB), e.g. a system including core areas consisting of natural or semi natural habitats, separated by a matrix of different levels of lower valued open areas. Thus the core-area populations are expected to become smaller and more isolated, in which case they might form a meta-population for wildlife species residing in fragmented environments (Hanski 1999), given that the matrix allows gene flow between sub-populations. For the CMB to be efficient, the matrix should be able to support either sink populations, or affordable passage between core populations.

Thus, land-cover composition of such matrices is crucial for the efficiency of the CMB. In contrast, Hodgson et al. (2011) concluded that the quality of the matrix is less influential than the size and availability of prime habitat. Different land cover types, ranging from natural/semi-natural non-habitat, to humanly disturbed areas, vary in their impact on the viability and connectivity of the matrix, depending on species. The threat or disturbance level of each cover type can be modeled into a continuous layer, to assess the probability of an area to serve as an effective corridor. The society for the protection of nature in Israel (SPNI) published a preliminary evaluation of corridors and passages in Israel, based on the INPA corridor map, and according to the physical properties of each such corridor (i.e., length and width, Gabay and Zanzuri 2019). Thus, the quality of the matrix, and the properties of the surrounding environment also play an important role in determining the quality of the corridor.

We propose to examine the efficiency of Israel’s CMB systems in maintaining connectivity between populations of wildlife species existing in natural habitat patches. For this purpose we suggest using a combined approach (As suggested by Krosby et al. 2015), including spatial analyses, wildlife activity monitoring and genetic analyses of populations of target species in natural habitats along the corridors, to estimate gene flow barriers and the isolation levels among populations. This will be, to the best of our knowledge, the first attempt to evaluate CMB performance using such an array of techniques operating on different levels of wildlife biology and ecology.

METHODOLOGY

*Study sites and CMBs evaluation*

We suggest using the Mediterranean region of Israel as the model system for this study. This is because it is representative of CMBs that include natural core areas that are highly fragmented in time and space (Fig. 1). The first step in evaluating the efficiency of these CMBs would consist of spatial analyses, identifying the different components of the CMBs comprising the system at the local and regional scales. For that we will apply spatial analysis tools with existing land use – land cover maps, as prepared and updated regularly by HaMaarag – Israel’s national nature assessment program, crossed with the corridor maps published by the INPA, and recently updated by the Society for the Protection of Nature in Israel (Gabay and Zanzuri 2019).

We will record the relative availability of different land cover types within each CMB system, divided into core areas, the type of ecosystem they consist of, and land uses in the matrix (such as plantations, agriculture, roads, infrastructure facilities). We will also map barriers (mostly linear infrastructure like roads and railroads, but also fences and other barriers) and obligatory passages crossing such barriers. The goal is to locate three CMB systems in the Mediterranean region, along the precipitation gradient.

All spatial analyses will be conducted in Qgis and ArcGIS software. These analyses will allow us to conduct a threat assessment (a combined effect of anthropogenic disturbances), in which the effect of human modified areas decays with distance (Levin et al. 2007). The decay function is probably not linear; however, it is impractical to predict how it behaves per cover type. In addition, different environments are probably affected differently by similar usages. Thus, we propose to apply a single decay function, following an exponential function with 50% decay at 100 meters, and asymptotic to 0 effect at 1000 meters from the edge of the disturbance/threat. Cover types will include (but are not restricted to) settlements, roads, agriculture, army camps and training areas, infrastructure, touristy locations, quarries and planted coniferous forests. Furthermore, we will apply landscape metrics analyses to estimate the severity of landscape fragmentation and evaluate the degree of expected isolation between natural patches ('landscapemetrics' package in R). This will eventually allow us to make quantitative testable predictions of the efficiency of any specific CMB section. Additionally, it will allow us to identify key areas in which to test such predictions, in defined CMBs.



Figure 1: An example of a land-use Land-cover map of the Mediterranean region in Israel. Green colors represent natural land cover, grey represents human modified areas. Dark green lines are EC borders after Rotem et al*.* 2015. Red lines represent critical EC sections for conservation (Gabay and Zanzury 2019).

*STUDY SPECIES*

Choice of species is of crucial importance, as the species need to fulfil several conditions (see Table 1). We realize that any choice made, would necessarily violate some of these conditions. To overcome this, we aim to sample several species from diverse taxonomic groups, representing a wide variety of wildlife with different ecological requirements and traits. In addition, in order to achieve novel understanding of corridors functionality, we aim to choose species representative of taxonomic groups that are largely ignored when dealing with ECs, at least in Israel (e.g. anything that is not a medium to large mammal).

Here we suggest a longer list of target species to avoid a situation in which a given species population does not provide enough samples *in-situ*. Ideally, five to six species from the list will be used in the study, depending on availability in the field. We will also consider 'control' species that presumably uses all CMB components freely. Suggested target species were chosen based on existing knowledge of species distribution and life histories from the literature and from consulting with experts. Table 2 summarize the suggested target species, the conditions (as specified in table 1) they comply with and the capture methods and seasons.

**Table 1** Conditions for choosing target species for the study and their rational.

|  |  |
| --- | --- |
| **Condition** | **Rational** |
| (1) Widespread and easy to obtain | Provide samples from multiple individuals |
| (2) Specific habitat requirements | Susceptible to fragmentation effects |
| (3) Short generation time | Allow multiple generations since CMB creation |
| (4) Well known genomes  | Allow better genetic analysis |
| (5) Indicative to the entire CMB system | Should be representative to all CMB components, either positively (present) or negatively (absent) |

**Table 2** Suggested target species, compliance with specified conditions, capture method and season. Ideally five to six species from the list will be eventually used in the study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Condition\***  | **Remarks** | **Capture method and season** |
| **(1)** | **(2)** | **(3)** | **(4)** | **(5)** |
| Cairo spiny mice *Acomys cahirinus* (Order: Rodentia) | √ | √ | √- | NA?? | √- |  | Live overnight trapping during spring and summer |
| House mice *Mus musculus* (Order: Rodentia) | √ | X | √ | √ | √- | 'Control' species | Live overnight trapping during spring and summer |
| Ocellated skink *Chalcides* *ocellatus* (Order: Squamata) | √ | √-- | √- | NA?? | √- |  | Active day-time search during spring |
| Common chameleon *Chamaeleo chamaeleon* (Order: squamata) | √-- | X | √- | NA?? | √- | 'Control' species | Active night-time search during spring and summer |
| Lebanon lizard *Phoenicolacerta laevis* (Order: squamata) | √-- | √ | √- | NA?? | √- |  | Active day-time search during winter-summer |
| Rüppell's snake-eyed skink *Ablepharus rueppellii* (Order: squamata) | √- | √-- | √- | NA?? | √- |  | Active day-time search during spring-summer |
| snake-eyed lizard Ophisops elegans (Order: squamata) | √- | √-- | √-- | NA?? | √- |  | Active day-time search during spring-summer |
| Fire salamander *Salamandra salamandra* (Order: Caudata) | √- | √ | √- | NA?? | √- | Local conservation status: CR A1c | Active night/day-time search in annual pools and streams during winter |
| Southern banded newt *Triturus vitatus* (Order: Caudata)  | √-- | √ | √- | NA?? | √- | Local conservation status: CR A1c | Active day-time search in annual pools and streams during winter |
| *Frontinellina frutetorum* (order: Araneae) | √ | √- | √- | NA?? | √- |  | Active day-time search during spring and summer |
| Walf spider *Pardosa subsordidatula* (Order: Araneae) | √- | √-- | √- | NA?? | √- |  | Active night-time search during spring and summer |
| *Argiope spp.* (Order: Araneae) | √- | √- | √- | NA?? | √- | Four species, depend on availability in the field | Active day-time search during spring and summer |
| Megarian banded centipede *Scolopendra cingulata* (Order: Scolopendromorpha) | √- | √- | √- | NA?? | √- |  | Active day-time search during spring and summer |

\* See table 1 for details.

√ presumably comply with specified condition.

√- presumably close to comply with specified condition.

√-- presumably partly comply with specified condition, but usually largely unknown.

NA information not available.

X does not comply with specified condition.

*SURVEYS*

After Identifying key CMB areas and performing pre field surveys, we will identify and decide on the final list of target species. We will then conduct a series of field surveys to collect samples. We are aiming to use three CMB systems in the study; each will include at least two core areas separated by a corridor with a matrix of anthropogenic development and activities. We aim to collect samples from approximately 30 individual animals from each target species in each core area, corresponding with 60 samples per species per CMB times three CMBs to a sum of 180 samples per species. The surveys will use standard sampling techniques, depending on the species (se also table 2):

1. **Rodents** will be caught using live animal traps (H.B. Sherman Trap, Ink. Tallahassee, FL, USA) baited with dry peanut snacks. Traps will be placed in the afternoon in sites specifically aiming at the target species. All traps will be visited first thing in the morning. All caught animals will be recorded and released on site. Target species will be handled for DNA samples (feces, swabs, or tissue) and released on site.
2. **Reptiles** will be caught using active searches. Specific search technique depends on the species and might include visual search, search under covers or night-time search with torches. Target species will be hand-caught and handled for DNA samples (feces, swabs, or tissue) and released on site.
3. **Amphibians** will be caught using active searches during the breeding season in specific aquatic sites known to support it. Target species will be hand-caught and handled for DNA samples (feces, swabs, or tissue) and released on site.
4. **Arthropods** will be collected using active search. Specific search technique depends on the species and might include visual search, search under covers or night-time search with torches. Individuals belonging to target species will be collected and removed from site for further DNA extraction.

Surveys' timing and extant will depend on species activity season and availability. As we aim primarily to understand genetic dynamics in the CMB system, surveys will target species specifically. As much as possible we will use non-invasive methods for obtaining the samples (using feces or slabs if applicable; e.g. Ketter-Katz et al. 2013).

**All live animal collection and handling will be performed under specific permits from both Israel Nature and Parks Authority, the University of Haifa Animal Ethics Committee and any other institutional approval as required.**

*GENETIC ANALYSIS*

In order to determine the level of separation/connectivity among populations, that may be caused by the barriers, and/or mitigated by the corridor-passage system, we will conduct population genetic analysis on our selected model species. To assess the genetic diversity DNA fingerprinting techniques will be applied, as their capabilities to reveal high levels of diversity at high resolutions. The selected species will be sampled for genetic materials, which will be analyzed using Restriction site associated DNA markers (RAD-sequencing) and/or microsatellites techniques, to identify difference in SNPs (single nucleotide polymorphism) and/or allelic composition related to genetic isolation. SNPs (single nucleotide polymorphisms), are the most abundant and dense type of genetic markers that provides a powerful DNA fingerprinting technique for any origin or complexity. Restriction site Associated DNA Sequencing (RAD-Seq) genotyping is a partial genome sequencing strategy that uses Illumina next-generation sequencing to simultaneously discover and score numerous (thousands) SNPs markers in a number of individuals (Etter et al., 2012). Microsatellite analyses are species specific and thus are less prone to foreign DNA contamination, hence tolerating non-invasive DNA sampling.

#### DNA SAMPLING

Genetic sampling will require samples from approximately 30 individuals from each population, summing up to approximately 180 samples per species (three CMBs, two populations per CMB, thirty individuals per population). Each collected sample will be inserted and stored in a vial filled with ethanol until DNA extraction. DNA will be extracted in the laboratory by standard extraction method using DNeasy Blood and Tissue extraction kit (Qiagen).

*RAD LIBRARY PREPARATION*

The RAD-seq procedure will follow Safran et al. (2016). In short, high molecular weight genomic DNA will be digested with *Eco*RI and *Mse*I restriction enzymes. Adaptors will be ligated to the sticky ends of the fragments, consisting on the priming sites for Illumina sequencing, following by barcode sequences that will allow unique identification of sequences from each individual. Various barcoded adaptors (Illumina) will be used, to allow sequencing of numerous individuals of a species in one Illumina HiSeq sequencing lane. Sequencing will be performed in available out campus companies (e.g. Weizmann Institute of Science, the Nancy and Stephen Grand Israel National Center for Personalized Medicine (G-INCPM); CD Genomics, Shirley, NY, USA, or The National Center for Genome Research, Santa Fe, NM, USA; Majorbio, Shanghai, China).

*MICROSATELLITES ISOLATION*

At least 10 microsatellites showing high number of alleles/locus will be tested for each species. The isolation of the various microsatellites and the design of the suitable primers, for species that have not been tested for microsatellite before, will be done by commercial company. The analysis will include PCR amplification of various microsatellites to be found. The F- primer for each microsatellite will be labeled with a florescent dye (6-Fam, Vic, Ned and Pet). Amplification products concurrently with LIZ 500 size marker (Applied Biosystems) will be visualized under a Florescence-Reader (Applied Biosystems).

*RAD data analysis*

We will use Burrows–Wheeler Aligner (BWA; Li and Durbin 2009) to align data for each individual against a better known large reference genome assembly (according to the species sequenced) to be known at time of analyses. To identify variant sites in an assembled sequence and to obtain genotype likelihoods for variable sites, we will use SAMTOOLS and BCFTOOLS software packages (Li et al. 2009). Based on these results, we will use a Bayesian model to estimate population allele frequencies (Gompert et al. 2012).

*Microsatellite data analysis*

Allele identification and genotyping will be determined directly from the chromatographs using Peak-Scanner software (Applied Biosystem). Mean number of different alleles (Na), number of effective alleles (Ne), observed and expected heterozygosity, and the fixation index Fis values, as well as analysis of molecular variance (AMOVA) procedure will be followed the methods of Michalakis and Excoffier (1996) and will be analyzed by GenAlEx software (Peakall and Smouse 2006).

*Genetic differentiation analyses*

The level of genetic differentiation between patches, and the estimation of the number of genetic subpopulations will be performed by a Bayesian clustering method (STRUCTURE). The analysis divided the samples into possible homogenous groups (sub populations) according to their degree of genetic similarity (STRUCTURE 2.3.4 admixture model; burn-in of 100,000 steps and 100,000 iterations; five replicates for each K) (Pritchard et al. 2000; 2010). The inference of the probable number of clusters will be extracted by the log likelihood for each putative number of populations (K), Ln P(D) = L(K), and by the delta K methods (Evanno et al. 2005), using the program Structure Harvester (Earl and vonHoldt 2012).

### **Synthesis**

A combined analysis of all the above will allow us to (a) obtain essential knowledge on CMBs functionality, and (b) provide detailed recommendations on planning land-use composition within CMBs in an optimal way, which will reduce negative impacts on both wildlife and stakeholders. While there is a myriad of specific variables that may affect CMBs quality and function, we believe there are some basic generalizations that may be inferred from a systematic evaluation, as we propose here. The immediate value from this work will be a reduction of collateral damage from land-use alterations, suffered mainly by natural ecosystems and wildlife.

However, farmers, entrepreneurs, municipalities and open landscapes managers would undoubtedly benefit in the short and long term from such knowledge-based management, by reducing conflict of interests and human-nature conflicts. Finally, we expect to see an increase in ecosystem services provided by CMBs following our recommendations.

#### PRELIMINARY RESULTS

A preliminary spatial analysis, based on layers of EC and critical corridor segments (Fig.1) was conducted, to determine how ECs compare to the overall conditions in Israel in terms of human activity and relative land cover types. The analyses revealed that ECs are less affected than the mean effect. 81% of the delineated ECs are covered by natural vegetation, compared to 67% of overall. In terms of overall threat, calculated using a weighted mean with an exponential decay function, threat level inside ECs are 0.98 compared to 1.77 in general (Fig. 2). However, within EC sections declared critical by ISPN, threat levels are similar to the overall level (1.73 compared to 1.77), indicating narrow corridors suffer relatively high threat levels.

Figure 2: Comparison of weighted threat levels in the Mediterranean region, all ecological corridors, and critical segments of ECs

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