Preventing the extinction of the Dinaric-SE Alpine lynx population through reinforcement and long-term conservation

Monitoring protocol for the Romanian source of Eurasian lynx population

A2. Assessment and selection of sites, and lynx, for live-capture from the Carpathian source population in Romania

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Focşani, September 2018

Report for the implementation of action A2

Realized in the frame of action A2:

Assessment and selection of sites and lynx for live-capture from the Carpathian source population in Romania

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Content

Foreword

The purpose of this document is to provide detailed information about the objectives, attributes, sampling design and data management necessary for the implementation of the action A2.

The goals of the A2 action are to determine the number of individuals that frequent the study area, and to identify the most suitable areas and micro-locations for live-capture of animals in Romania.

Since this monitoring activity will be maintained for the entire project life, data collected will be useful to evaluate the threshold of number of individuals that can be removed from each area without compromising the population and to evaluate the effects of lynx removal for translocation purposes on the source populations (D1 action).

This document is required by the need to ensure a technical framework for the LIFE Lynx project team.

Objectives and attributes

The main *goals* of this action (A2. Assessment and selection of sites and lynx for live-capture from the Carpathian source population in Romania) are:

1) to collect spatial information useful to identify *the most suitable areas* and *micro-locations for live-capture of animals* in Romania.
2) to determine how many lynx individuals are present in the study areas during each winter of the whole project period. The *abundance* calculated before, during, and after catching period will be useful also to estimate the effects of lynx removal on the local population (action D2).

To achieve the *goals* described above, three main *objectives* have been identified: *evaluation of distribution*, *abundance*, and *activity of lynx* inside the study areas.

The *attributes* and/or *variables* that will be measured during the sampling surveys, for each objective, have been described below.

Distribution

The most basic information about a species is its presence in a certain location. During the entire project period we will collect spatial information from both direct and indirect lynx signs. Data gathered, supported by a constant sampling effort, will be useful not only to describe where the species occurs but also to evaluate the trend of the frequency of occurrence of the signs over time among, and within, the study areas.

The spatial information obtained, (namely lynx trails followed during snow tracking surveys, and the location of camera traps with lynx registers collected during camera trapping surveys) will be used to establish the suitable areas and micro-locations for live-capture of individuals.

Abundance

To assess the abundance of lynx in the study areas, the *minimum number of individuals* and *family groups* in a given area will be determined by combining the information resulting from camera trapping and snow tracking surveys, and genetic data.

Family groups are defined as a unit composed by the mother with one or more kittens. This association is particularly frequent before the mating season, during the early to mid-winter (November-February).

Three relative indexes of abundance will be reported every winter season for each study area.

One index is calculated as number of camera trap photos/videos of lynx per camera trap day. The camera trap day is defined as each 24-hour period that a camera trap is functioning to capture photos/videos of lynx.

The other two *relative abundance* are calculated as the:

- *number of lynx tracks per number of transects,*
- *number of lynx tracks per total km of transects travelled.*

Although these measurements do not provide precise information on how many lynx live in each study area, they can provide trend of the lynx occurrence over time in the study areas.

Activity

Lynx activity will be recorded by camera traps each 24-hour day. The data gathered will be useful to determine not only when an individual crosses a specific site but also the number of times it crosses the same site during the winter (*frequency of events, latency time, and period between two consecutive events*). The activity information will be fundamental to establish suitable sites for live-capture of animals.

Table 1. Objectives, variables, sampling strategy and methods

Objectives	Variables	Sampling strategy	Methods
distribution	min. range occupied	systematic and opportunistic approach	snow tracking, camera trapping, genetic analysis
abundance	min. no. of families	systematic and opportunistic approach	snow tracking, camera trapping, genetic analysis
	min. no. of individuals	systematic and opportunistic approach	snow tracking, camera trapping, genetic analysis
	index of tracks abundance	opportunistic approach	snow tracking
	index of videos abundance	systematic approach	camera trapping
activity	frequency of events	systematic approach	camera trapping
	period between two consecutive events	systematic approach	camera trapping

Sampling design

The following paragraphs contain detailing information regarding:

- the study areas, the sampling procedure, and the selection criteria (*sampling details*)
- techniques and survey details (*survey protocol*).

Sampling details

Study areas

The project area covers a large expansion of the Eastern Romanian Carpathians, and, potentially, an area located in the Central Romanian Carpathians. Whitin this range, seven pilot study areas have been selected to conduct lynx surveys and monitoring activities (Fig. 1). Surfaces of the study areas will vary between 400 and 700 sq.km, which will increase the chances of several lynx or lynx families living in the area. The limits of the study areas will be defined by squares of 10x10 km, using the EEA reference grid (https://www.eea.europa.eu/data-and-maps/data/eea-reference-grids-2).

The characteristics of the study areas were established following these guidelines:

- ✓ the European Environmental Agency reference grid was used to support the EU policy regarding the request of standardization of data collection at European scale (Annoni, 2004),
- ✓ the surface of each study area (ranging from 400 to 700 km^2) was set according to Zimmermann et al., (2013), who suggests, that the surveys have to be conducted over areas large enough to guarantee robust results from a biological and statistical point of view,
- ✓ the squares were selected in areas where we assumed lynx had higher probability of detection. This approach is suggested by Boitani and Powell (2012) to maximize sampling efficiency and save resources,
- ✓ moreover, the study areas were selected as a compromise between maximizing the genetic diversity between lynx and minimizing the logistic weaknesses (travel costs, inaccessibility, lack of support and acceptance of the project goals by the hunting administrations).

Due to large surfaces of the study areas and limited personnel and technical equipment (i.e. camera traps, boxtraps), survey, monitoring and trapping activities will follow a sequential order, with 1 to 3 study areas being monitored simultaneously over the period of 1 to 2 years (i.e. Year 1 and 2 – Study areas 1-Putna, 2-Bacau, 3-Vintileasca; Year 3-4 – Study areas 4-Vanatori-Neamt, 5-Calimani and 6-Hasmas or 7-Fagaras). Ideally, with the help of volunteers, more than 2 areas will be monitored simultaneously.

To ensure a good monitoring and focused effort, each study area will have a different importance over the course of the project years. To express the importance, study areas will be considered as *primary*, *secondary* and *additional*.

Primary study area – is defined as an area in which the majority of the effort will be concentrated. A study area can be primary for the monitoring activities and secondary for trapping activities, and vice versa.

Secondary study area – is defined as an area in which activities will be performed sporadically, in order to gather information that will ease the monitoring and trapping activities when the area will become of primary importance.

Additional study area – is defined as an area in which activities will be performed only if no trapping success was registered in the other study areas. The importance of an additional study area (primary or secondary) is to be decided considering the situation at that time.
After an initial monitoring of the entire study area, specific locations, where high densities of lynx signs were observed, will be selected for boxtraps placements.

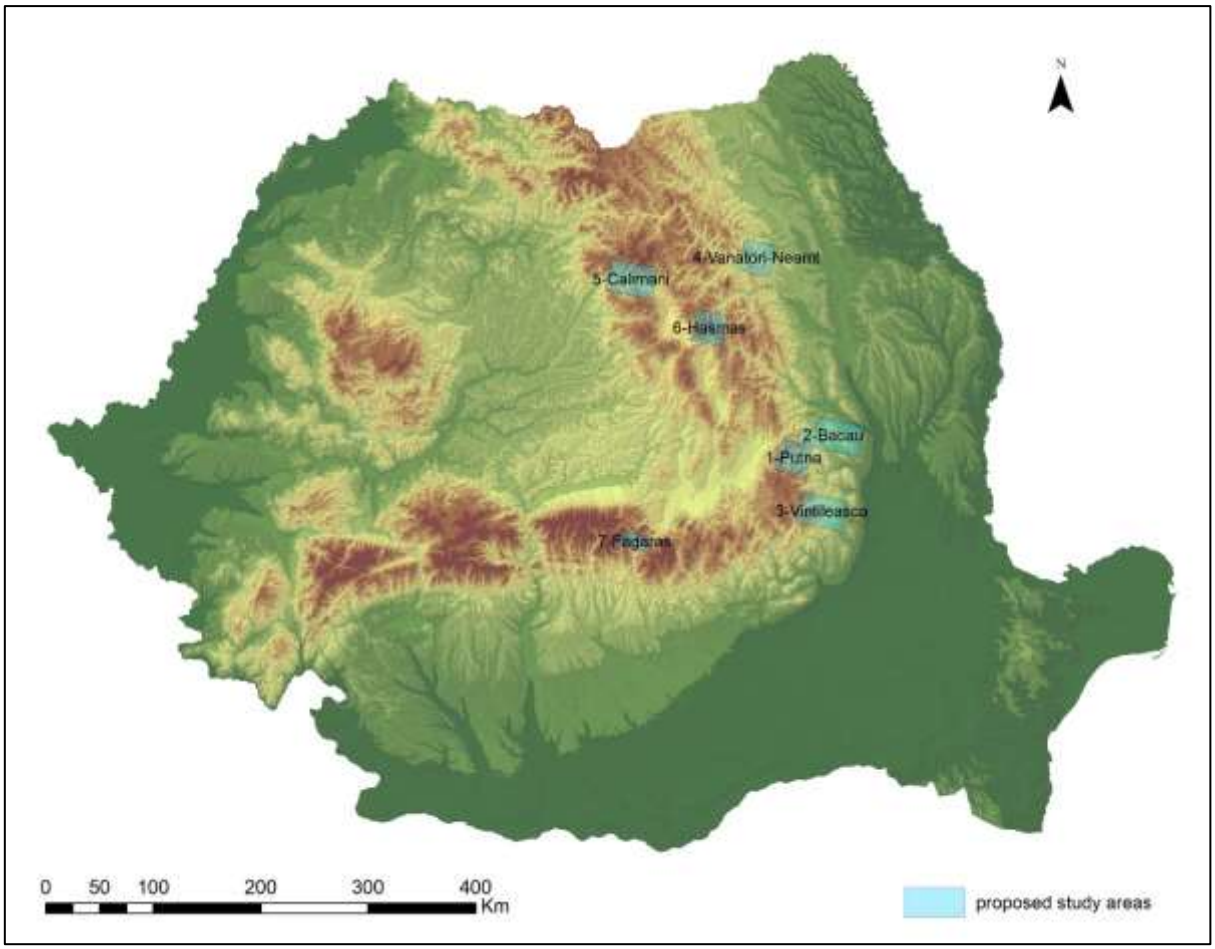

Figure 1. Distribution of the suggested study areas.

Survey, monitoring and trapping time-frame

(See annex for time-table)

Scenario 1

Year 1 (November 2017 - May 2018)

1- Putna –primary study area, monitoring activities;
2- Bacau –secondary study area, monitoring activities;
3- Vintileasca –secondary study area, opportunistic survey.

Year 2 (mid-September 2018 – mid-April 2019)

1- Putna – primary study area for trapping;
2- Bacau – primary study area for monitoring;
3- Vintileasca – secondary study area for monitoring and trapping.

Year 3 (mid-September 2019 – mid-April 2020)

1- Putna, Bacau, Vintileasca – secondary study areas for trapping;
2- Adittional study areas (1 or 2 study areas, will be chosen at the time) – primary study area(s) for monitoring.

Year 4 (mid-September 2020 – mid-April 2021)

1- Adittional study areas (1 or 2 study areas, will be chosen at the time) – primary study area(s) for trapping;
2- Putna, Bacau, Vintileasca – secondary study areas for trapping, some active boxtraps will be maintained if needed.

Year 5 (mid-September 2021 – mid-April 2022)

1- Adittional study areas (1 or 2 study areas, will be chosen at the time) – primary study area(s) for trapping;
2- if needed, lynx trapping will occur in one of the known study areas; to be decided based on the results from previous years;
3- post lynx-removal monitoring activities could initiate if time and obtained results (i.e. number of trapped and translocated lynx) allow it.

Year 6 (mid-September 2022 – mid-April 2023)

1- If needed, lynx trapping will occur in one or two of the known study areas; to be decided based on the results from previous years;
2- post lynx-removal monitoring activities in all study areas

Scenario 2

Year 1 (November 2017 - May 2018)

1- Putna –primary study area, monitoring activities;
2- Bacau –secondary study area, monitoring activities;
3- Vintileasca –secondary study area, opportunistic survey.

Year 2 (mid-September 2018 – mid-April 2019)

1- Putna – primary study area for trapping;
2- Bacau – primary study area for monitoring;
3- Vintileasca – secondary study area for monitoring and trapping.

Year 3 (mid-September 2019 – mid-April 2020)

1- Bacau – primary study area for trapping;
2- Putna, Vintileasca – secondary study area for monitoring and trapping.

Year 4 (mid-September 2020 – mid-April 2021)

1- Putna, Bacau, Vintileasca – secondary study areas for trapping;
2- Adittional study areas (1 or 2 study areas, will be chosen at the time) – primary study area(s) for monitoring.

Year 5 (mid-September 2021 – mid-April 2022)

1- Adittional study areas (1 or 2 study areas, will be chosen at the time) – primary study area(s) for trapping;
2- Putna, Bacau, Vintileasca – secondary study areas for trapping, some active boxtraps will be maintained if needed.

Year 6 (mid-September 2022 – mid-April 2023)

1- If needed, lynx trapping will occur in one or two of the known study areas; to be decided based on the results from previous years;
2- post lynx-removal monitoring activities in all study areas

Sampling strategies

An *active sampling* approach will be adopted for the entire period of the project. In the *active sampling*, the data collection comes from personnel adequately trained in appropriate data collection, methods, and analytical procedures (Breitenmoser et al., 2006).

Whitin the active sampling approach, we will rely on two different strategies: *systematic sampling* and *opportunistic sampling*.

Systematic sampling ensures that the effort is spatially and temporally planned. In our case, camera-trapping will follow a systematic sampling. The number and distribution of stations, the camera trap working mode, and the survey period have been previously set (as reported within the camera trapping survey protocol) and will be maintained for the whole study period. This procedure is necessary for obtaining a precise measurement of the population attributes.

Conversely, in the *opportunistic sampling* procedure, the effort is not spatially and temporally planned (snow-tracking). This sampling procedure is applied only to increase the amount of information obtained, when and were needed.

Information from tourists or local people (*passive sampling*) will not be taken into account, unless it has been verified and confirmed by the experienced personnel.

Criteria for analysing and interpreting collected data

The interpretation of lynx monitoring data will be conducted following the SCALP criteria (Molinari-Jobin et al., 2012).

This approach allows the assignment of each record of lynx presence to three different categories (C1-C2-C3). Each category has a different degree regarding level of trustworthiness, in terms of accuracy of species identification.

The definition of the three categories are reported here below as described in Molinari-Jobin et al. (2012):

C1: Confirmed "hard facts", verified and undisputable records of lynx presence such as (1) dead lynx, (2) captured lynx, (3) good quality and georeferenced lynx photos (e.g., from camera traps), and (4) samples (e.g. excrements, hair) attributed to lynx by means of a scientifically reliable analysis.

C2: Records confirmed by a lynx expert (e.g. trained member of the network) such as (1) killed livestock or (2) wild prey, and (3) lynx tracks or other assessable field signs.

C3: Unconfirmed category 2 observations (kills, tracks, other field signs too old or badly documented, where however the description conforms to a lynx sign) and all observations such as sightings and calls, which by their nature cannot be verified.

Criteria to define "experienced personnel"

A person is considered "experienced" if he/she has know-how in field data collection (i.e. qualified in recognising and interpreting signs left by the species in the field).

Professionals and volunteers forming a lynx-monitoring network are part of this group, but they can be also professional staff such as game wardens or forestry guards, who are regularly trained for this task.

Survey protocol: techniques and survey details implemented for monitoring the local lynx population

Snow tracking

The main aims of snow tracking activity within LIFE LYNX project are: to identify which sectors of the study area are frequented by lynx, to evaluate the number of individuals and family groups, and to identify the most suitable areas and micro-locations for live-capture of animals. In each study area, except for the sectors characterized by a high level of human activity and presence (agriculture areas, and settlements), a *set of systematic transects* have been identified. The *set of systematic transects* is designed to allow the operators to cover the largest amount of area possible, and to maximise the probability of intercepting lynx trails. Besides the standard transects, occasionally, additional transects will be surveyed, with the aim of increasing the chances to collect sufficient biological samples for genetic analysis.
Transects were selected following the main and secondary mountain ridges, and the valleys, paths and forestry roads which are generally used by lynx for their movements. Each transect has a length of not less than 7 km and ideally should cover the altitude range of the area, from the bottom of the valley up to the mountain ridge, to maximize the probability of crossing a lynx trail. The transects will be performed when a continuous ground coverage is present to allow us to detect, and track, a lynx trail as much as possible, without interruptions.

Snow tracking surveys are conducted 24-48 hours after a snowfall. This interval is necessary for the animals to make extensive movements and maximize the probability that tracks are intercepted by the operators. The same snowfall will be used until its physical conditions allow the recognition of the tracks and the approximate estimate of their age. Snow tracking should be avoided in presence of deep and partially melted snow because it negatively affects the track recognition.

Once the track is intercepted, it should be followed as far as possible (in both directions) in order to: count number of individuals, record lynx movement and signs (to ascertain the lynx use that area) and to allow the collection of fresh samples (excrement, urine, hair), which are useful for the genetic analysis.

Snow tracking follows an opportunistic sampling strategy, and the amount of effort is strictly dependent on snow cover conditions. However, in the primary study areas, the team of operators (two/three crews, with each crew being composed by at least two people, of which one must be an expert personnel) will attempt to perform the set of transects, three times, every winter, once per bimester (Nov-Dec; Jan-Feb; Mar-Apr).

Camera trapping

The main aims of camera trapping activity within the LIFE LYNX project are: to figure out the minimum number of individuals and family groups in each study area, to detect specific used sites (paths and marking points), and to evaluate their visiting rate.

Camera trapping follows a systematic sampling strategy, with the number of stations for each study area, camera settings and working period being previously planned.
However, at micro-location level, the exact place of the station will be selected as to maximize the probability of recording lynx videos. As Stergar and Slijepčević (2017) reported, three different type of sites will be selected to set the devices:
1- in proximity of the shelter (generally used as marking point);
2- at the potential movement corridors (path, forestry road, ridge, valley);
3- close to the fresh kill site.

Although two devices at each station are often recommended for the individual recognition, based on the unique coat pattern (Breitenmoser et al., 2006; Kubala et al., 2017; Stergar and Slijepčević 2017), we planned to use only a single device at each station to increase the number of stations and the probability of detecting lynx.

A number of 3-4 camera traps will be set in each square of primary study areas. No rigourous established number is planned for the secondary study areas.
The devices will work 24h a day. All the stations, except for those at the kill sites, will be monitored on a monthly basis. As reported in the Lynx camera trapping guidelines (Stergar and Slijepčević 2017), every month the crew will visit the station to:
1) check if the camera trap is still at the location;
2) check if the camera is shifted or moved;
3) change SD memory card;
4) check and replace the batteries in case of low capacity.
If no photos or videos are collected after two months, the operators can decide to move the camera at another location.
No attractants will be used to induce animals to visit the camera trap stations.

The equipment is composed of 50 devices belonging to different models (Moultrie_ M80XD; Moultrie_ M-880; Moultrie_M-880i; Moultrie_M-990i; Moultrie_ M-990i_Gen2; Cuddeback Black Flash; StealthCam). The devices have different flash technology: from infrared (IR) to black IR. The camera traps will be set to collect videos (30 sec length) to obtain not only information of individual recognition, but also about its behaviour. Hour (winter time) and date will be associated to each video. Camera delay will be set to the minimum possible time.

We will perform a systematic intensive camera trapping activity (with regular checking of camera traps) from the beginning of November to end of March or April. We will remove the cameras will be removed from the field in order to minimize the chances of being stolen, as in that period human movements in the study areas increases.

Genetic analysis

Non-invasive genetic analysis is one of the most widely used techniques in the study of animals characterized by high mobility and living in low densities, such as the lynx.

The main aims of genetic analysis within this specific action of the LIFE LYNX project is to obtain information on: identification, sex, and relatedness of the individuals. Based on this information, it is possible to measure some demographic parameters, such as: distribution, minimum number of individuals, population structure by gender, survival and dispersal rates.

Collection of non-invasive genetic samples follows an opportunistic sampling strategy and relates to the field work, mainly the snow tracking activity. The target is to collect at least 30 genetic samples during regular field work.

Samples used for genetic analysis are lynx scat, hair, urine, saliva, blood and tissue.

Information on procedures for collecting samples and preserving the genetic materials are reported in the specific manual "Collecting lynx non-invasive genetic samples. Instruction manual for field personnel and volunteers" (Skrbinšek, 2017).

Data management

Particular attention will be given to data storage. All aspects of the monitoring program will be carefully collected and archived. Data collected in the field will be transcribed into an electronic format that can be stored both on an external hard disk and on the laptops of all four technicians. The electronic format takes both the form of a simple Excel-based database with standardized column headings and pre-defined data entry codes and a GIS database (shapefile).

The database will be regularly backed up and the backup copy stored on the external hard disk.

The database is structured in two parts, each having two sections (effort and lynx presence):

1. Ground survey database
2. Camera trapping database

1. Ground survey database

The ground survey's database is composed of two parts: the first section contains information in terms of number of kilometers (effort) patrolled by the crews (exclusively travelled on foot); the second section contains information on lynx presence (videos, signs).

The information reported in the first section:

- ✓ the identification of the study area;
- ✓ the identification of the square 10x10 km crossed by the transect;
- ✓ the identification code of the transect;
- ✓ the date on which the inspection was carried out;
- ✓ the bimester when the inspection was carried out;
- ✓ the amount of km of transect patrolled in each square;
- ✓ the physical condition of the terrain;
- ✓ additional information;
- ✓ name of the operators.

The information reported in the second section:

- ✓ the identification of the study area;
- ✓ the identification of the square 10x10 km where the sign was collected;

- ✓ the identification code of the sign;
- ✓ the barcode in case the sample was collected for the genetic analysis;
- ✓ the coordinates (decimal degree, UTM, WGS 84);
- ✓ the date on which the inspection was carried out;
- ✓ the estimation of freshness of the sign (< 7 days; from 8 days to 1 month; >1 month);
- ✓ the estimation of deposition time of the sign (month);
- ✓ the bimester when the sign was collected;
- ✓ the category of lynx sign collected (scat, lynx trace, urine, hair, etc.);
- ✓ If the sign was found following the lynx trace and was associated with that, the identification code of the lynx trace is reported;
- ✓ length of the part of lynx trace associated with the square 10x10 km;
- ✓ number of individuals observed;
- ✓ identification of the carcass at the kill site;
- ✓ additional information;
- ✓ names of the operators;
- ✓ information related to the data collection (for diet): binary code (0,1);
- ✓ information related to the data collection (for genetic analysis): binary code (0,1);
- ✓ assignment to one of three categories according to the following SCALP criteria.

2. *Camera trapping database*

The camera trapping's database is composed of two parts: the first section contains effort's information in terms of number and location of the stations, number of devices, and working days; the second section contains information on lynx presence (photos and videos).

The information reported in the first section:

- ✓ the identification of the study area;
- ✓ the identification of the square 10x10 km where is located the station;
- ✓ the identification code of the station;
- ✓ Latitude;
- ✓ Longitude;
- ✓ site's description 1: shelter/forest road/footpath/outroad;
- ✓ site's description 2: kill site/lynx sign/no previous information;
- ✓ the identification code of the camera trap;
- ✓ the identification code of the SD card;
- ✓ date of starting;
- ✓ date of ending;
- ✓ number of working days;
- ✓ total number of videos collected;
- ✓ human disturbance (0,1);
- ✓ total number of empty videos;
- ✓ note: additional information;
- ✓ operators_1: responsible of setting camera;
- ✓ operators_2: responsible of checking camera.

The information reported in the second section:

- ✓ the identification of the study area;
- ✓ the identification of the square 10x10 km where the sign was collected;
- ✓ the identification code of the station;
- ✓ the identification code of the camera trap;
- ✓ the identification code of the video;
- ✓ Latitude;

- ✓ Longitude;
- ✓ Date;
- ✓ Hour;
- ✓ Minutes;
- ✓ Species: lynx;
- ✓ Number of indviduals;
- ✓ Temperature (in Celsius degree);
- ✓ Snow cover (0: absent, 1: < 20 cm, > 20 cm);
- ✓ additional information;
- ✓ Link with the video;
- ✓ Operators_1.

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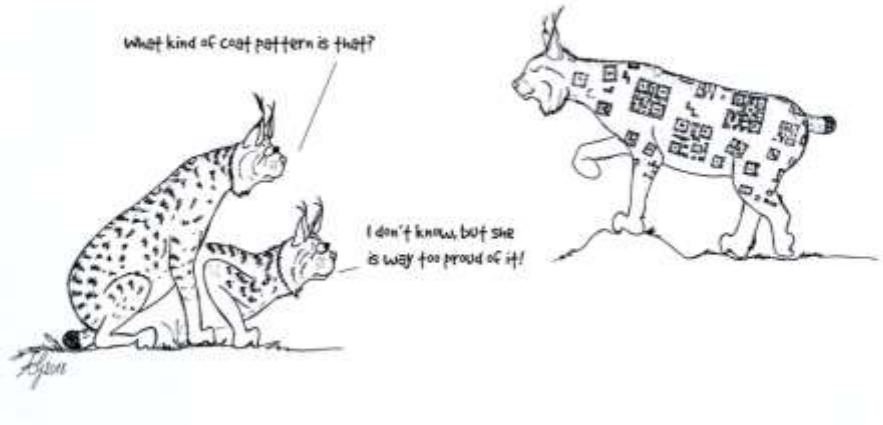
What kind of coat pattern is that?
I don't know, but she is way too proud of it!