MARMOT ALPINE PROJECT

Fieldguide

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INTRODUCTION

The Alpine Marmot Project began in 1990 when the Vanoise National Park staff wanted to learn about the habits of the alpine marmot so they could resolve conflicts between farmers and marmots. Farmers believed that marmots were causing damage to their hayfields. Discussions were initiated between the marmot research team and the organization responsible for the management and protection of wildlife. La Grande Sassière National Nature Reserve was chosen as the study site because it had many accessible marmots, as well as a cozy chalet for the researchers.

At the time, knowledge about the alpine marmot was rudimentary. Therefore, early studies focused on the behaviors, social structure and spatial structure of the species. Researchers determined that the alpine marmot is a social species living in extended families on a well-defined territory. Each family is composed of a pair of monogamous dominant individuals, and a variable number of subordinate individuals of both sexes. These early results came from research on the physiology of hibernation, behavior, life history traits and population dynamics. Major research themes include: Evolution of monogamy; Evolution of social structure; Senescence (aging); Effect of climate change on marmot interactions.

We have learned that the alpine marmot is one of very few monogamous mammals. However, like the monogamy observed in birds, social monogamy is rarely associated with genetic monogamy. In the alpine marmot, the dominant social couple can maintain that status for many years. Although genetic monogamy is dominant, it is not absolute. In fact, our research indicates that approximately 20% of litters contain young that are not those of the dominant male, and 10% of young are not genetically related to the dominant male. We are therefore looking for reasons that extra-pair paternity exists.

We know that the alpine marmot experiences an aging process, or senescence. Although pervasive, senescence is an incredibly complex and variable process, varying from one species to another and even from one individual to another. We are exploring the origin of this variation, including the role of genetic factors, environmental factors, conditions at birth or social context. What evolutionary theories could explain these variations? The first results on this topic have shown marked differences between the sexes. Because of their status and their role within the family group, males tend to age earlier than females. For example, dominant males must both monopolize reproduction and defend their territory against intruders. As they age, their mass decreases, which reduces their ability to maintain their dominant status. Such a phenomenon is not observed in dominant females. Researchers are also interested in the potential role of sociality in the process of senescence, especially regarding reproductive success. That is, are there social factors that might affect the reproductive success of individuals, as measured by litter size, weight of each of the young at birth and total litter weight?

Finally, climate change is currently the greatest threat to biodiversity. Evidence for climate change includes an increase in average temperatures and an increase in extreme climatic events (floods, storms, drought ...). On a global scale it is difficult to predict the impact of these changes on the functioning of ecosystems, partly because these changes are not uniform throughout the world. However, as with coral reefs and mangroves, mountain ecosystems are particularly vulnerable to climate change, with changes in the Alps being easily measured. For example, the average temperature increase in the Alps is twice as high as that observed in the rest of the northern hemisphere. An increase in winter precipitation and a decrease in recent summer precipitation were also observed. This causes a decrease in the duration and height of the snowpack throughout the Alpine Arc, as well as a faster shrinking of most glaciers and a decrease of alpine permafrost.

The consequences of climate change affect many plant and animal species. Climate change causes habitat fragmentation, changes in the distribution of species and the disappearance of the most sensitive species. Some species are already showing changes in phenotypic traits (for example, in body weight) and in phenology (temporal sequence of events in the life cycle), as well as some downsizing or even local extinctions. Alpine species are specialists, well suited to their mountain climate. Therefore, many of them will be especially sensitive to climate changes in their environment, making them good candidates as sentinel species.

In such an environment, ecosystems and alpine species should benefit from careful observations made by researchers trying to understand and predict the impacts of climate change on population dynamics. In this context, the Alpine Marmot Project has a major role to play, from both a scientific and societal point of view, to ensure the protection of threatened species and habitats. One of the main objectives of the Alpine Marmot Project is to study the ecological consequences of climate change on alpine populations, using the alpine marmot as a model. To do this, we will search for links between climate parameters (temperature, precipitation, etc.) and population dynamics among the marmots.

PRESENTATION OF THE BIOLOGICAL MODEL - THE ALPINE MARMOT

Latin name	*Marmota marmota*	
Common name	Marmotte alpine	
Lenght	45-68 cm	
Weight	2.2-6.5 kg	

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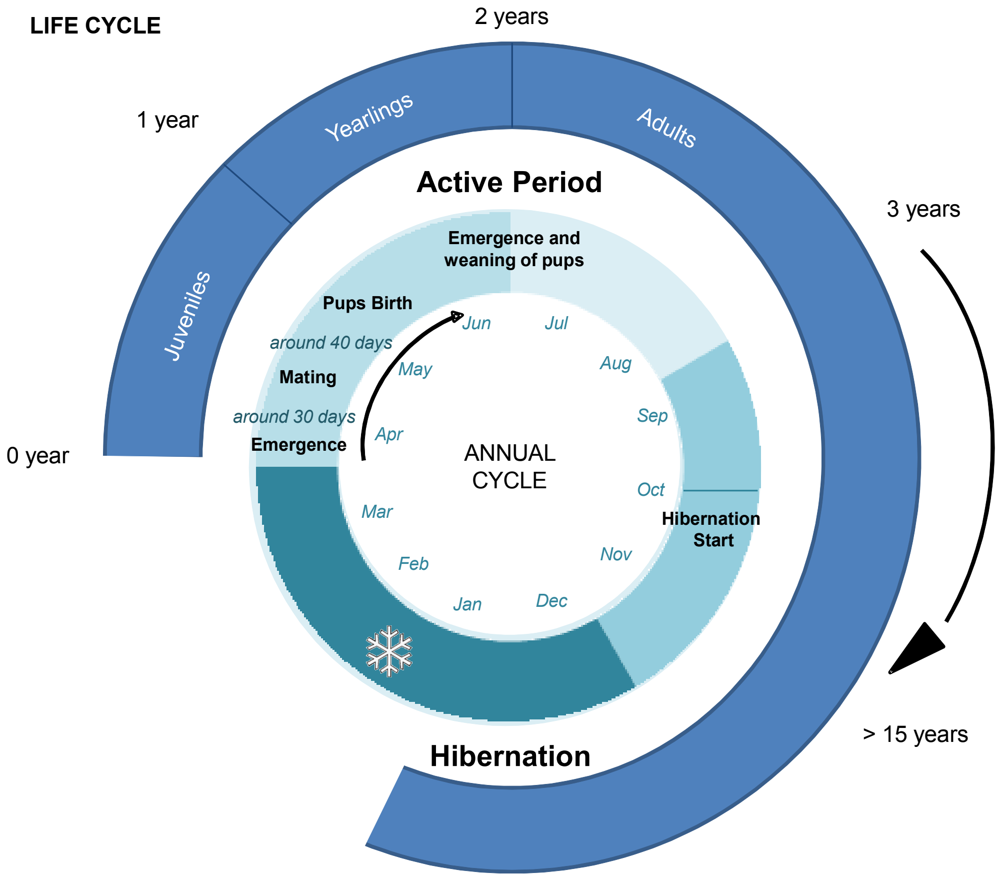
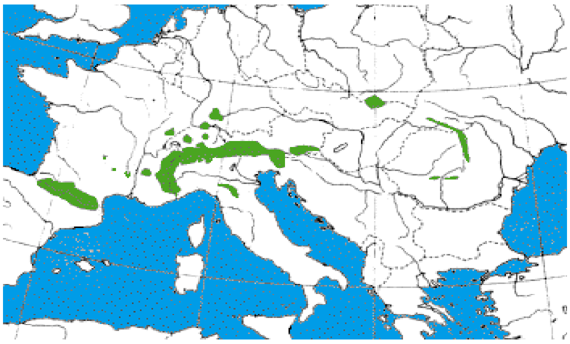
Annual and life cycles



Distribution	The alpine marmot is found throughout the Alps, from France in the west to Slovenia in the east (Map 1). In France, it is also present in the Pyrenees and the Massif Central where it has been introduced.

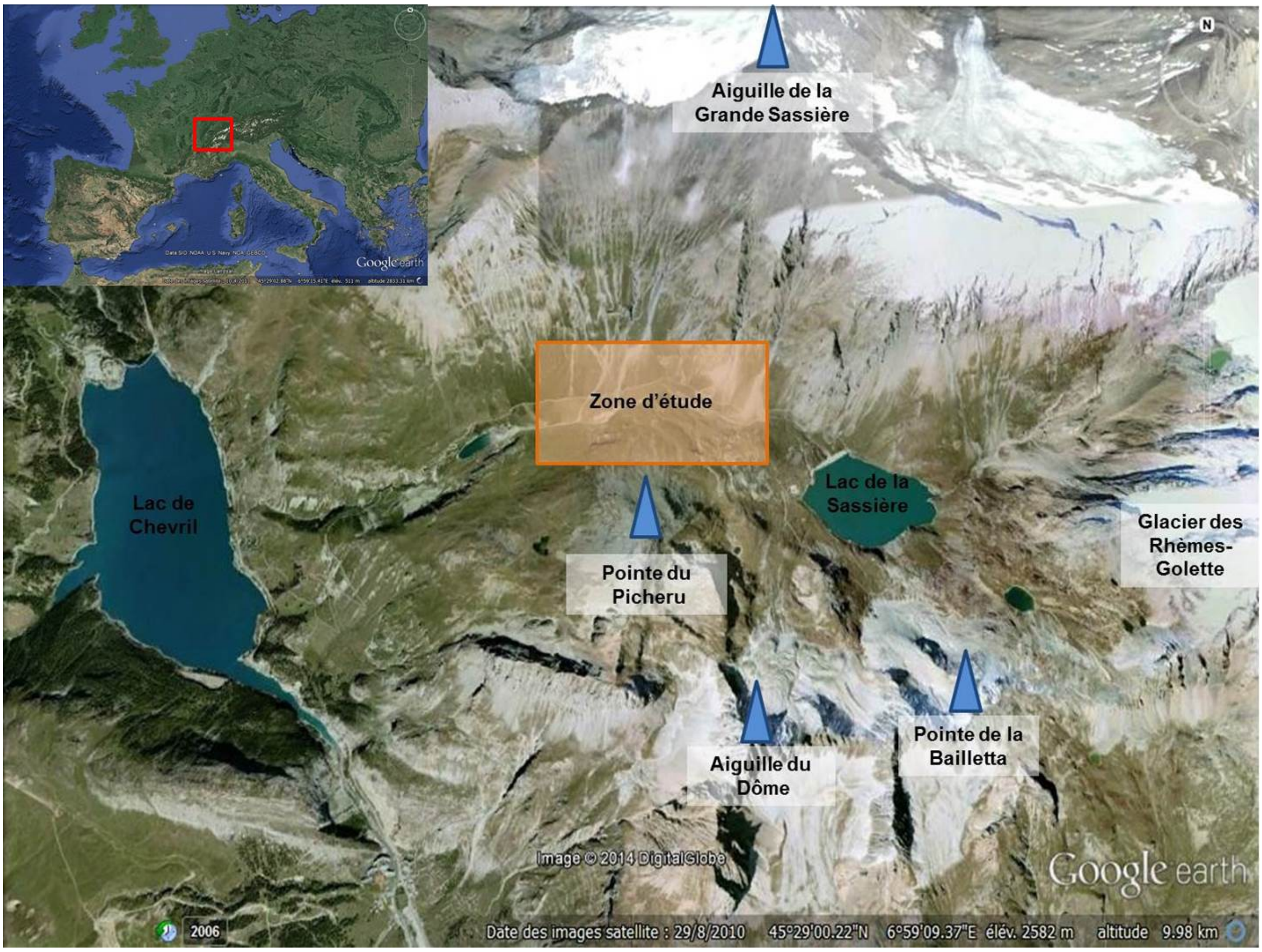


Map 1: Current distribution of the alpine marmot (green)

Habitat	The alpine marmot inhabits lawns, subalpine and alpine meadows between 800 and 3000 meters in altitude.
Social organisation	The social structure of the marmot is organized around the family. A family group consists of a pair of dominant adults, a variable number of male and female adult subordinates, juveniles and pups. Only the dominant pair reproduces. A marmot family occupies an average area of 2.5 ha; the boundaries vary little from one year to another. A territory consists of a main burrow, secondary burrows and latrines.

PRESENTATION OF THE STUDY SITE -
LA GRANDE SASSIERE NATIONAL NATURE RESERVE

Located in the valley of the Haute- Tarentaise, in the town of Tignes, the Grande Sassière National Nature Reserve was created August 10, 1973 to compensate for loss of a reserve where a ski lift would be built as part of the Tignes ski complex. Since then, the Reserve has been managed by the National Park of Vanoise. The Grande Sassière Reserve covers 2,230 hectares, bounded on the north by the Aiguille de la Grande Sassière, on the south by the Aiguille du Dome and on the east by the Glacier of Rhêmes-Golette (Map 2).



Map 2: Study site location

With an alpine climate and the extremely varied soils that occur between 1800 and 3750 meters in altitude, the Grande Sassière National Nature Reserve is home to diverse animals (fauna) and plants (flora). Famous for its large population of marmots, it contains substantial populations of several other species characteristic of the Alps, shown below.


Photo 3 :Ibex


Photo 4 :Chamois


Photo 5 :Golden eagle


Photo 6 :Bearded vulture

Plants present on the reserve are not outdone, because there are more than twenty rare species (photos 7, 8, 9 and 10), twelve protected at the national level and eight at the regional level.


Photo 7 :Edelweiss


Photo 8 :Alpine columbine


Photo 9 :Alpine Panicaut


Photo 10 :Androsace

Despite comprehensive protection of the site, a by-law allows grazing to continue in the lower part of the valley. Therefore, cattle grazing occur during the warm season (photo 11).


Photo 11: Cow of the race Abondance in alpine meadows

SCIENTIFIC MONITORING OF THE POPULATION OF ALPINE MARMOTS

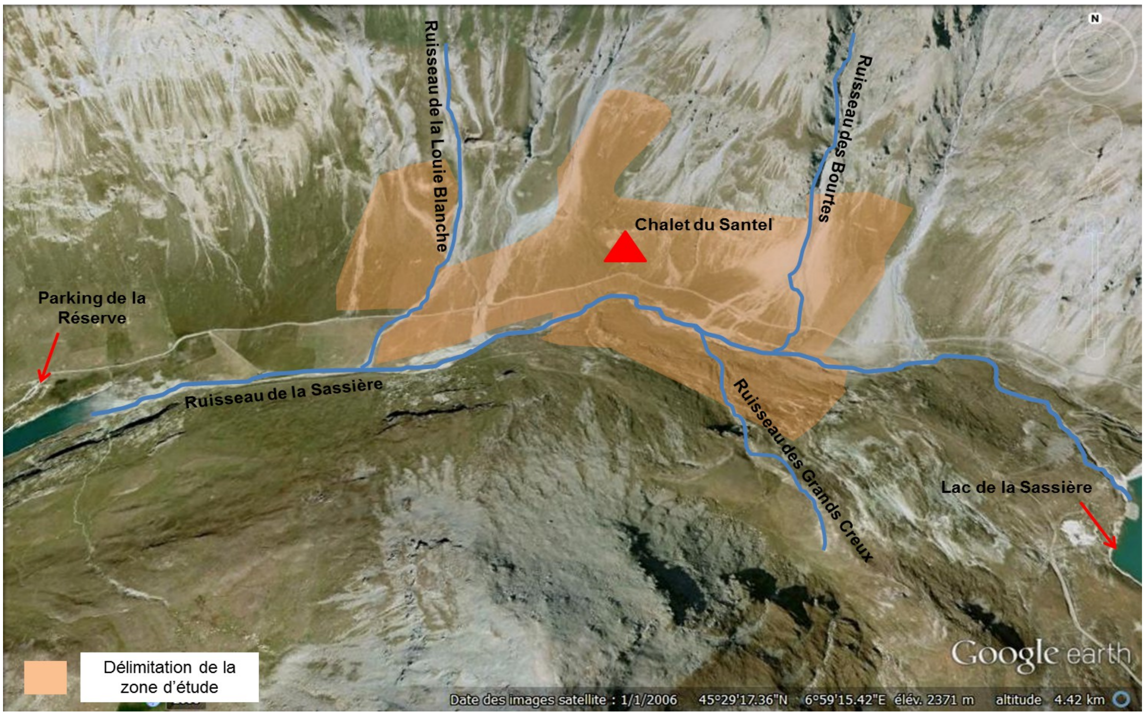
History

Since 1990, researchers have followed 26 marmot families each year, and have identified more than 1300 individuals. By early 2014, the database contained information for more than 3,500 captures. In the database, researchers recorded the identity of the captured marmot, its sex, age, any outstanding morphological characteristics and any previous samples taken, such as blood or fur. This crucial information is still far too rare for wildlife, even though it would provide answers to many questions about how marmots relate to their environment.

Starting in 2012, Earthwatch volunteers have joined us and helped to collect and process data.

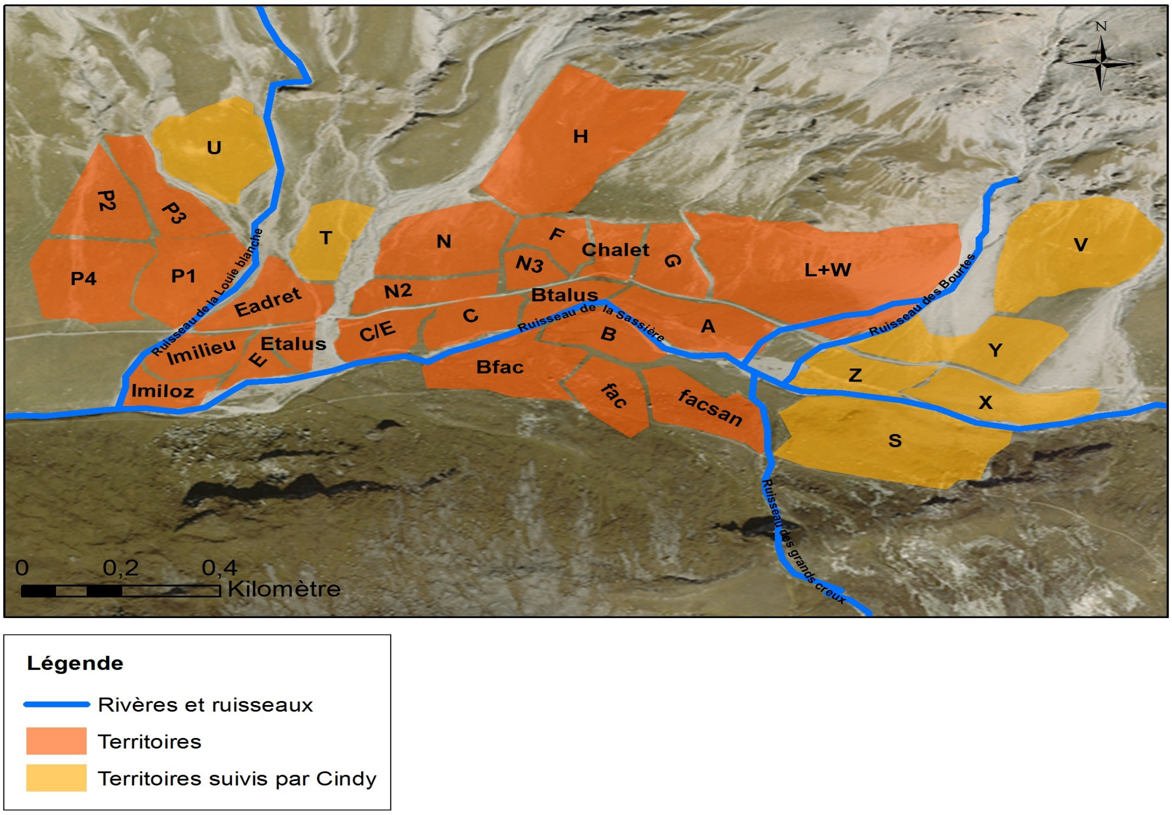
Study area

The area where we study the marmots is located in the western part of the Grand Sassière National Nature Reserve, at an altitude of 2350 m and an area of about 40 ha that includes the Santel chalet. Four streams go through the area, one from east to west and the other from north to south, partitioning the study zone. Two other streams flowing north to south help determine the boundaries of the study zone.



Map 3: Current boundaries of the study area

Within the study area, the spatial structure of each family's territory is precisely known, and is updated annually. The population seems to have reached its saturation space: between 1990 and 2013, only six new territories were created by splitting existing territories



Map 4: Map of current territories

In each territory, the entrance to a main burrow is often on a mound of dirt (photo 12). The burrow consists of a main entrance and peripheral secondary entrances.

Photo 12: Main entrance to a burrow from the outside

Activity 1 - CAPTURE PROTOCOL - Recognition of burrows and territories (map of marmot territories).

CAPTURE-MARK-RECAPTURE PROTOCOL

Objective

The most important reason for this activity is to allow researchers to track individual marmots throughout their life. It allows us to follow three important demographic parameters: survival, reproduction and dispersal of individuals. In addition, it tracks the dynamics of the population.

A second benefit of capturing individuals is that researchers can make various measurements on them and take biological samples, which are essential for obtaining information about the growth rates of individuals, their health status, physiological condition, genetic characteristics and kinship relationships.

Marmots are captured each year using the same protocol during the breeding season, that is to say mid-April (when marmots begin to emerge from hibernation) until mid -July (when all the pups have emerged). This is the only period when marmots can be captured. Beyond that time, marmots have no interest in the dandelion bait and cannot be captured.

Step 1 – Capture

Capturing marmots with traps

Marmots are captured using traps with two entrances (Figures 1 and 2). The traps are placed near the main burrow in each territory.

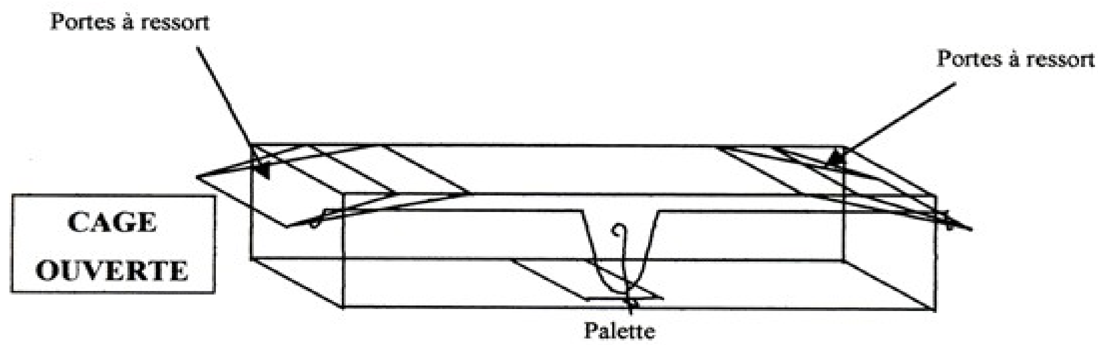


Figure 1 : Open trap

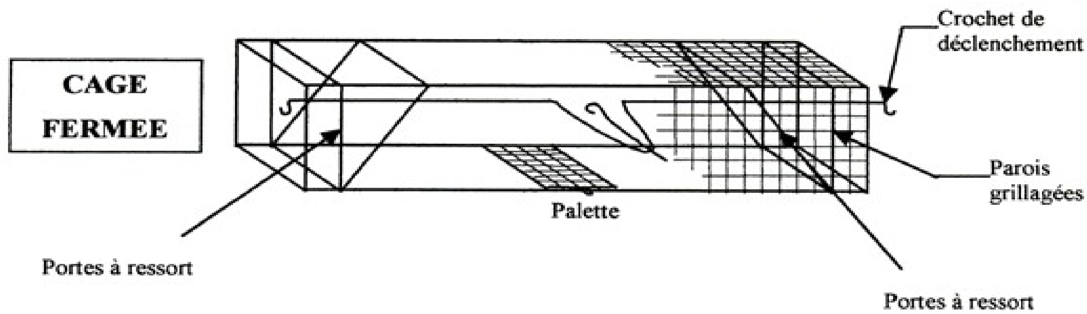


Figure 2 : Closed trap

Every morning the traps are opened and baited with dandelions placed on a platform in the middle of the cage. Upon entering the cage to eat the dandelions, marmots walk on the platform and trigger the closing of the entrance doors.

Activity 2 – CAPTURE PROTOCOL - Collect dandelions

Activity 3 – CAPTURE PROTOCOL - Bait the traps with dandelions

Once set, the traps are checked every half hour under normal conditions (mild weather). If weather conditions deteriorate (rain, snow), the traps are visited immediately, and any captured marmots are returned to their burrow. Marmots are put to sleep as soon as they are captured.

Activity 4 - CAPTURE PROTOCOL - Monitoring traps

Capturing marmots by hand

This method is used for marmot pups leaving their burrow the first few times. A researcher keeps watch on a primary burrow to see when the pups emerge, and then captures them by hand (photo 13 and 14). This technique is extremely effective (up to ten pups caught per day) and usually the entire litter can be caught in a short time, thereby maximizing data quality and minimizing stress on the pups. However, it is only effective on pups that are still "naive", that is to say, within a few days of their first outing into the open air, but while still staying close to the burrow where they were born.

Photo 13 et 14 : Method of capturing pups by hand.

Step 2 – Mark

Identification

When a new marmot is captured, it is given a unique identification that it keeps throughout its life. To accomplish this, researchers use three ways of tagging each marmot. The first method involves injecting a transponder (photo 15 and 16) under the skin of the animal. No bigger than a grain of rice, each transponder has a unique alphanumeric code up to 16 characters long. It is placed under the marmot’s skin between the shoulder blades. These are passive chips whose reading is done by reflection using a receiver placed within 30 cm of the individual. To obtain all the recorded data, the individual must be re-captured.

Numbered metal ear rings (photo 17 and 18) are placed on the right ear of females and the left ear of males. The number can be read using a telescope if the marmot is less than 20 meters away. In most cases, the marmot must be recaptured for the researchers to read the number. The average lifespan of these rings is more than two years.
Finally, colored ear rings are also used (photo 19 and 20). The first is an ear ring, placed on the left ear of dominant females and the right ear of dominant males. These colored rings allow researchers easily to distinguish dominant marmots from subordinates.

Spray paint (safe for pets) provides a second method for using color in studying marmots (photo 21). The paint covers a portion of the marmot’s body, and is extremely effective for visual recognition at a distance. On the other hand, the paint stays on the marmot for less than two months.

Besides the ear rings and paint used for identification, each new marmot is given a name.

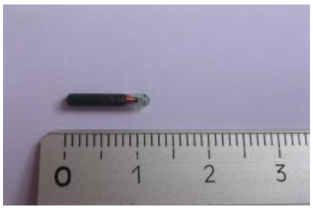


Photo 15: Transponder


Photo 17 : Attaching a metal ear ring

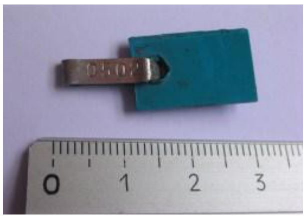


Photo 19 :Colored ear ring

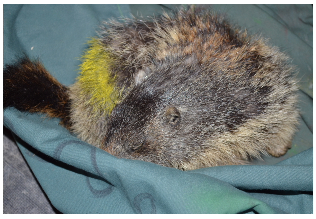


Photo 21 :Individual marked with colored spray paint


Photo16 : Inserting a transponder


Photo 18: Individual with a metal ear ring


Photo 20 :Individual with a colored ear ring

Activity 5 - CAPTURE PROTOCOL - Report all individuals with a colored mark (Data sheet 1)

Characterization

Sex

The sex of each captured marmot is determined. With the animal in hand, it is easy to distinguish the male from the female. Just look at the distance between the anus and the urethra. In males the distance is large (photo 22), while in the female the urethra seems "stuck" to the anus (photo 23).

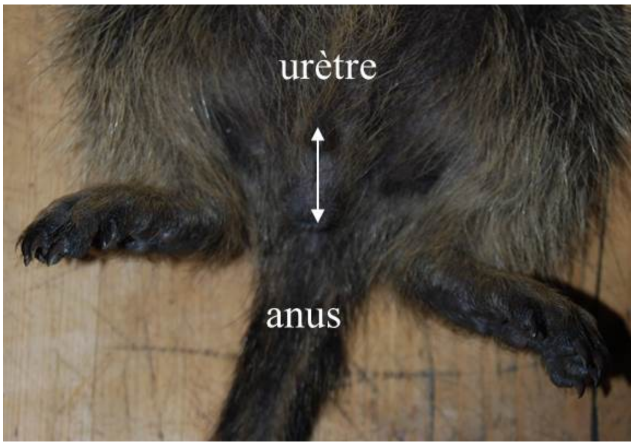


Photo 22 : Anogenital distance in males

Photo 23 : Anogenital distance in females

Age

Marmots are divided into four age categories for the project:

	Marmot pup	Yearling	Sub-adult	Adult
Age	From birth to 6 months	6 to 12 months	12 to 24 months	More than 2 years
Mean height (cm)	24	38	46	48
Average weight (g)	524	1860	3100	3719
	 Photo 24 : Marmot pup	 Photo 25 : Yearling	 Photo 26 : Adult	

Social status

When individuals are caught, their social status is determined. It is easy to distinguish between the dominant and subordinate individuals. A dominant male has a prominent scrotum (photo 27) and a dominant female has visible and well developed teats (photo 28). In addition to these features, the dominant individuals develop hairless scent glands in their cheeks.

Photo 27 : Scrotum well developed in the dominant male

Photo 28 : Teats well developed in dominant female

Biometric Measures

First, each individual marmot is **weighed** with the kind of spring scale used for weighing fish (photo 29). Then, several **biometric variables** are determined, including: total length, measured using a tape measure (photo 30); length of the front legs (photo 31); length of rear legs (photo 32); length of the mandible (photo 33); width of the head at the cheekbones (photo 34); and width of the pelvis (photo 35). These measurements are made with a vernier caliper.

Photo 29 : Weighing

Photo 30 : Total lenght

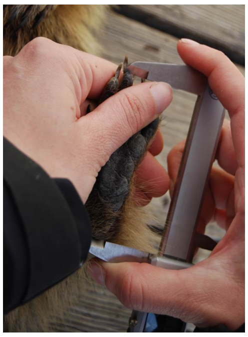


Photo 31 : Front leg length

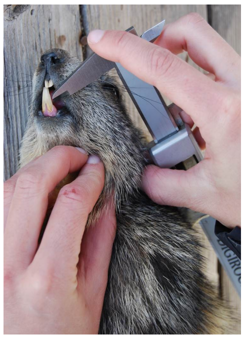


Photo 33 : Mandible lenght

Photo 34 : Head width

Photo 32 : Rear leg length

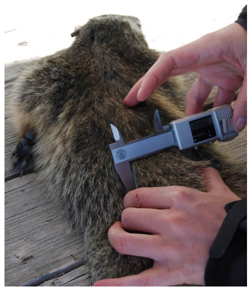


Photo 35 :Cheekbone width

Biological samples

Four biological samples are taken: blood (photo 36); fur (photo 37); skin tissue (photo 38) and odorous secretions (photo 39).

The drawn blood sample is used for measuring levels of hormones, isotopes and various molecules and metabolites, as well as for chemical characterization of the immunological profile of the individuals.

Samples of fur and skin are used in genetic analyses and in hormonal and isotopic assays. Genetic analyses are crucial for establishing the genealogy of individuals and the genetic structure of the population.

Three types of odorous secretions are collected: anal, mouth and cheek. These samples will allow us to characterize the different odorant molecules that marmots secrete to communicate with each other or to recognize individuals.

Photo 36 : Blood collection
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Photo 37 : Sampling fur
©C et D Favre-Bonvin

Photo 38 : Sampling skin
©C et D Favre-Bonvin

Photo 39 : Sampling odorous secretions
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Step 3 – Release

Once all measurements and sampling are completed, the animal is placed in a quiet area in the capture bag until it wakes up. Then it is carried in the bag to the area where it was captured, and released at the entrance to the main burrow.

Photo 40 : Marmot released into its burrow after measurements were made and samples taken

Activity 6 – CAPTURE PROTOCOL - Release

PROTOCOL FOR OBSERVING MARMOT BEHAVIOR

Objective

The social structure of the alpine marmot revolves around the family group. A family group consists of the following categories of individuals: a dominant couple that monopolizes reproduction (or tries to); subordinate individuals who are sexually mature but don't reproduce; immature marmots one year old; and pups of the year. The alpine marmot is highly social. In particular, marmots of the same family group cooperate with each other to defend their territory and to rear the young.

Knowledge of the size and composition (age, sex and kinship) of family groups is necessary for assessing the benefits and costs associated with characteristics of family groups, including survival and reproduction of individuals. For example, these observations allow us to quantify the costs of reproductive competition between dominant and subordinate individuals, but we can also evaluate how the presence of subordinates affects the reproductive success of various dominant couples.

Monitoring the size and composition of family groups

The first set of observations is intended to establish the number, social status, age and sex of the individuals constituting a family group, namely:

- The identity of the two dominant individuals;
- The number of subordinate males two years or older
- The number of subordinate females two years or older;
- The number of subordinate males one year old;
- The number of subordinate females one year old.

For this morning, in pairs, using binoculars and a telescope, you will observe a marmot family and identify the individuals who leave the main burrow. Each individual is identified by his or her ear tags or color of dye on its back. The sex of individuals is determined by metal rings on their ears. Males have metal rings on their **left** ear, and females on their **right** ear.

Dominant individuals are identified using colored ear rings. Dominant males wear this colored ring on their **right** ear, dominant females on their **left** ear. The dominant couple may also be identified by their particular territorial behavior: marking their territory. They tend to regularly mark their territory by rubbing their cheeks against "objects," such as stones or earth mounds. These observations also help us determine the physical boundaries of each territory. The age of subordinate individuals is determined by their size.

Activity 7– COUNTING MARMOTS PROTOCOL (Data sheet 2)

Recording reproduction milestones

This second type of observation aims to determine the timeline of activities related to reproduction. It is especially important to establish the exact date when the pups leave their main burrow for the first time, usually between mid-June and mid-July. These data allow us to obtain the date of emergence of marmots for each family group, and the number of marmots produced by each family.

To obtain this information, you will work in pairs using binoculars (10x50) and a telescope (20x60). From mid-June to mid-July, all burrows in each territory are observed every morning and every afternoon.

Activity 8 – REPRODUCTION PROTOCOL – Date of emergence (Data sheet 3)

Activity 9 – REPRODUCTION PROTOCOL – Counting pups (Data sheet 4)

Activity 10 – REPRODUCTION PROTOCOL – Video of the pups

Behavioral experiments - Testing the phenomenon of “Dear Enemy"

In addition, behavioral experiments are conducted to better understand the social organization of the family and the interactions between individuals and family groups.

The alpine marmot is a so-called territorial species, that is, the members of a family group live within and defend a defined space, chasing away foreigners who enter the family’s territory. The dominant male and female use scented secretions produced by their cheek glands to mark the boundaries of their territory, as well as their main burrows. These secretions signal to intruders that the space is already occupied.

However, defending a territory is costly in time and energy. It is therefore expected that an individual reacts less strongly to the intrusion of a dominant neighbor who has its own territory and thus represents only a limited threat. By contrast, the intrusion of an unknown dominant individual, who may or may not have its own territory, represents a significant threat. This adjustment of an animal’s reaction, depending on the level of threat, is known as the phenomenon of "Dear Enemy".

Photo 41 : Jugular gland

Behavioral experiments are being conducted to test whether, in fact, the reactions of the dominant male and female differ when they detect the scent of a dominant neighbor, as compared with the scent of an unknown dominant individual. To explore this question, two glass tubes are impregnated with the smell of a dominant neighbor or the smell of an unknown individual. The scented tubes are presented to the dominant individuals of each nearby family. During each experiment, the reaction of the dominant individuals to each tube is filmed for later analysis.

Photo 42: Placing the scent of a foreign individual in a test tube

Photo 43: Placement of olfactory tubes on a study area

Photo 44: Observation of individuals being studied

Activity 11 – DEAR ENEMY PROTOCOL – Behavioral experiments

Once behaviors are recorded on videos, they are analyzed.

Activity 12 – DEAR ENEMY PROTOCOL – Video Analysis - Step 1

Activity 12 – DEAR ENEMY PROTOCOL – Video Analysis - Step 2

Activity 12 – DEAR ENEMY PROTOCOL – Video Analysis - Step 3

Activity 13 – DEAR ENEMY PROTOCOL – Saving videos

WEATHER MONITORING

Objective

Twenty years after the first Earth Summit in 1992, the Rio+20 Conference reaffirmed that climate change is the greatest threat of the 21st century. The alpine environment is among the most threatened ecosystems (IPCC 2007; European Environment Agency, 2009), and the changes in climate observed in the Alps are unprecedented. Evidence of major climate change includes:
(1) Recent temperature increases more than twice the average observed in the northern hemisphere. Average temperatures in the Alps could rise by 3°C to 6°C by the year 2100
(2) A decrease in the duration and thickness of the snowpack across the Alpine arc,
(3) An increase in the frequency and intensity of extreme weather events (rainstorms, droughts).

In addition, mountain species are specialized for living in their alpine environment. A consequence of this specialization is their low capacity to adapt to changes in their environment. In agreement with theoretical predictions, the population of alpine marmots in the Grande Sassière Nature Reserve has shown a decline in the number of young produced during the last 20 years, a result that is consistent with smaller winter snowpacks linked to climate change.

In this context, researchers need to understand the impact of climate change, a major challenge from both a scientific and societal point of view, to ensure protection of threatened species and habitats. The "Alpine Marmot Project" is the only long-term program for monitoring a mammal on the metropolitan french territory, led by the National Center of Scientific Research (CNRS) and a university, and provides a unique opportunity to provide answers to current climate questions. To obtain these answers, it is essential not only to continue to obtain data characterizing the population of alpine marmots, but also to obtain climate data that characterizes the environmental conditions experienced by alpine marmots.

The weather station

To monitor the weather, a weather station is being installed close to the Santel chalet (photo 45). A device approved by the French weather agency, Météo France, will record the following parameters:

- Speed and wind direction,
- Solar radiation,
- Air and soil temperature
- Relative humidity,
- Amount of precipitation,
- Duration of precipitation,
- Amount of snowfall.

The energy required to run the station will be provided by a solar panel. Information will be recorded as recommended by Météo France, with the data integrated over an appropriate duration of time to each individual parameter. Data will be sent daily to the laboratory computer servers in the Department of Biometrics and Evolutionary Biology.

Temperature and brightness recorders

Since 2011, temperature and luminosity at entrances of main burrows have been recorded hourly using the device, “HOBO Temperature/ Light Data Logger 64K - UA- 002-64" (photo 46). These are placed in a hole at the entrance of the main burrow (photo 47) of each family group, and they record temperature and brightness hourly for 14 months. These data provide information on local climatic conditions experienced by marmots, and indirectly on the dates each family group begins and ends hibernation.

Photo 45: Model weather station planned for the study site

Photo 46: Device for recording temperature and brightness hourly

Photo 47: Placing the device in hole

Activity 14 – WEATHER MONITORING PROTOCOL - Step 1 - Removing device (Data sheet 5)

Activity 14 – WEATHER MONITORING PROTOCOL - Step 2 - Setting up device (Data sheet 5)

MONITORING THE ALPINE ECOSYSTEM

Objective

Monitoring the alpine ecosystem is a way to learn about phenology, which is the timing of periodic events that are determined by seasonal variations in climate. Indeed, the life cycles of plants and animals are not only directly related to seasonal changes in temperature and daylight, but also are highly dependent on weather conditions. The weather data will help us measure the impact of climate change on different stages of the life cycle, such as flowering or fruiting in plants or reproductive stages in animals.

In the context of climate change, extreme environments, such as alpine ecosystems, will be especially sensitive to these changes. It is therefore important to monitor the changes in the life cycles of alpine species in response to these climate changes. In this context, several plant and animal species are being monitored weekly to measure responses within a species, but also to compare the variations in these responses among species.

Activity 15 - PHENOCLIM PROTOCOL (Data sheets 6 et 7)

Activity 16 - PHENOPIAF PROTOCOL (Data sheet 8)

Activity 17 - PHENOZOO PROTOCOL - Monitoring grass frog (Data sheet 9)

Activity 18 - PHENOZOO PROTOCOL - Monitoring green dock beetle (Data sheet 9)

PRECAUTIONS TO TAKE WHEN IN THE FIELD

Stay on the path and away from marmot territories

Although familiar, marmots are still wild animals. To minimize interference with their daily activities, please stay on the paths. Do not park near the territories.

To avoid disturbing the marmots, use binoculars and a telescope when you want a closer view.

Follow these recommendations even when doing activities that are not directly related to marmots (Phenoclim, installing a monitoring device,...). For each activity, try to minimize the amount of time you spend in the territories.

Look carefully at the names of territories and their locations to make sure you are following the right family group.

Be very vigilant when making your observations, and accurately record the name and location of the territory where you are working. Whether you are catching marmots, making observations, performing experiments or recording climate parameters, it is crucial to record where you carried out these activities.

To achieve this goal will require rigorous concentration and accurate note-taking.

How to Relate to Tourists

Many tourists frequent the reserve. Some may ask you about your presence and your activities in the reserve. Feel free to explain what you are doing and why.

PRECAUTIONS REGARDING SNAKES, INSECTS AND STINGING PLANTS

To minimize snake and insect bites, it is advisable to wear high shoes and be vigilant in looking where you are planning to put your hands and feet. For example, do not put your hands anyplace where you have poor or no visibility (holes, stones ...). These precautions will help you avoid any possibility of bites or stings. Do not climb on boulders, where many venomous snakes may live (photo 48). Be careful when you walk around the cabin, where many stinging plants are present (photo 49).

Photo 48 : Aspic viper

Photo 49 : Common nettle

Sunburn, dehydration, hunger

Beware of sunburn at high altitudes. To prevent sunburn, always use sunscreen with a high number that blocks all or most of the sun. You can also get bad sunburn from sun reflecting off snow. As you go higher in the mountains, it becomes easier to get sunburned, even on cloudy days! It is therefore essential to protect your eyes and face with a cap or visor to avoid sunburn.

Wear appropriate sunglasses that protect against UV light and completely cover your eyes.

Especially during sunny days, drink water or other liquid regularly so you do not become dehydrated.

Remember to make a snack the night before to take into the field with you in case you become hungry during an activity. When you are going to be outdoors, always bring a bag or backpack with you containing warm clothing (that is, gloves, hat, scarf, rain jacket, fleece, an extra pair of socks ...). Mountain weather conditions often change very quickly and you need to be prepared for sudden cold or rain.

Steep slopes, slippery footing, rocks...

Remember to wear footwear that is designed for mountain use. In wet and snowy weather, the ground can be slippery. If you experience problems, ask a member of the team for walking sticks.

AT THE CHALET -
COMMUNICATION PROTOCOL

Objective

The Alpine Marmot Project is intended primarily as a scientific project, but at the same time, it is also a participatory project. To inform and involve the general public, as well as those who already care about the mountains, we plan to publicize our activities through our website and our facebook page:
http://projetmarmottealpine.org/
https://www.facebook.com/thealpinemarmotproject.

Using these two means of communication, we will share our research and the discoveries that result. The general public often believes that research is abstract, daunting and inaccessible to them. These media outlets provide a way for us to highlight the Alpine Marmot Project and to make our findings understandable to everyone. We will do this using short reports, summaries, photographs and non-technical language. These sites can help us to publicize our project and its research results. We can also use the sites to raise funds, thus helping to ensure that the Alpine Marmot Project continues for the long term.

Principle

To do this, we plan to summarize our scientific papers so that anyone interested can understand the results.

In addition, at the end of each week, we would like to publish a short summary of that week's field activities, including photographs.

We also would like to prepare a document about the project for Wikipedia, which would further publicize our research.

Activity 19 – COMMUNICATION PROTOCOL – Photo reporting

Activity 20 – COMMUNICATION PROTOCOL – Updating the facebook page

Activity 21 – COMMUNICATION PROTOCOL – Popularization of scientific articles

PROCESSING DATA FROM THE FIELD

Objective

As part of our research, it is essential to keep track of all data collected in the field because these data are used by all research programs and by all researchers and students. Therefore, after each field day, it is crucial to transfer all the data recorded in the field onto a computer. These data form the basis for answering all the biological questions about the alpine marmot.

Principle

Data about captured marmots are saved in two files: the "Capture" file, and the "Family structure" file.

The file named "Capture YYYY" contains all the data on each individual caught in a given year. Each line of the file refers to an individual captured on a given day. Each column lists all the information recorded on the capture sheet, which was filled out in the field.

The file named "YYYY Family structure" contains all the data relating to the composition of each family group of marmots during a given year. Each sheet represents a given territory, and each line of each sheet corresponds to a particular year of the monitoring

.

Activity 22 – DATA ENTRY PROTOCOL – Capture file (Data sheet 11)

Activity 23– DATA ENTRY PROTOCOL – Family structure (Data sheet 12)

TREATMENT OF BIOLOGICAL SAMPLES

Preparing hair samples

Objective

Hair taken from captured marmots is used for extracting DNA. These DNA samples are used in many analyses, such as in identifying the marmot's parents, which then lets us determine the genetic relationships among individuals in a family.

During reproduction, each parent transmits one copy of its genes (DNA sequence) through its gametes (reproductive cells). [to simplify: in the nucleus of almost every cell, DNA is structured in chromosomes. Chromosomes are thus an association of 2 copies of several DNA sequences = genes. In gametes, there is only one copy of these several DNA sequences]. Although a parent transmits all of its genes to its offspring, each parent contributes only one of its two copies of each gene. During formation of the egg cell by fusion of the male gamete and the female gamete, a diploid cell is created. (A diploid cell has the full amount of DNA, the 2 copies of genes; in this case, half comes from the male parent and half from the female parent.)

For example, if the X gene to the father was homozygous Xa/Xa (exactly the same version of the gene X = DNA sequence, at the same position) and Xb mother homozygous Xb/Xb (exactly the same version of the gene X as well, but with a small variation compared to the gene X of the father), then the child will be heterozygous Xa/Xb (for the same gene X, he got both versions!), because each parent has given a copy / allele of the gene X. The genotype of the child is the result of mixing a portion of the genotype of the father and the mother. Knowing the mode of transmission of genes and alleles (Mendelian), it is possible, by examining the genes of individuals, to determine the relationships of kinship between them.

In all animals, certain portions of DNA will encode genes (like a code that will be read and result on the synthesis of a special protein), and other portions of the same DNA will not. Among these non-coding sequences, some nucleotide (DNA unit) sequences will be repeated. The number of repeats is highly variable from one individual to another (between 5 and 50 repetitions, depending on the individual). These repeated sequences are called "microsatellites". The number of microsatellite repeats, characteristic of an individual, is inherited in the same manner as the alleles of a gene.

After obtaining the genotype (all the genes, which means every alleles = copies of every genes!) of each individual, and the number of repetitions for several microsatellite genotypes of different individuals, (e.g., the genotypes of pups and of the dominant male and female), the results are compared to determine genetic relationships within the family.

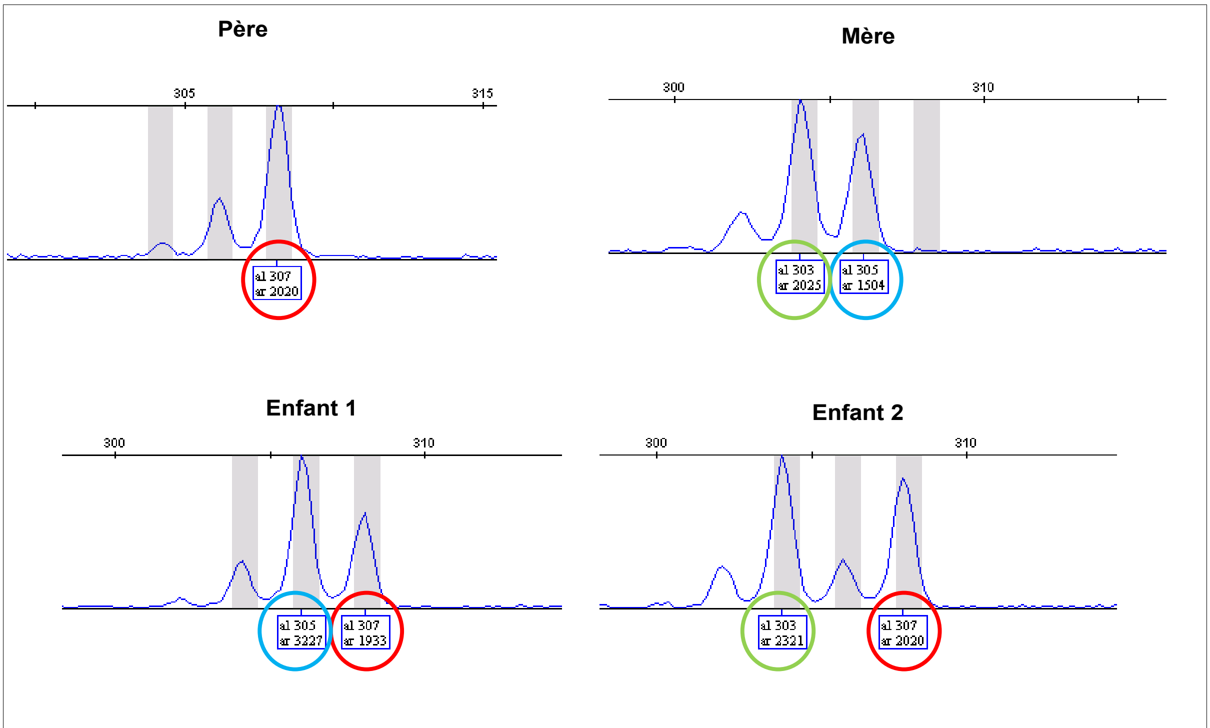
Principle

The genotype of every marmot is done using DNA. For this marmot project, DNA is extracted from the root bulbs of hair samples (remember, in almost every cells, there is a nucleus which contain DNA!). The DNA is purified by destroying other components of the fur root bulbs, such as tissues, protein and peptides, and then separating the DNA from these other substances.

Each microsatellite have flanking sequences (short parts before and after the microsatellite sequence), and the different individuals to study have them in common for a particular microsatellite. Primers corresponding these flanking sequences are then added to start hybridization with the DNA previously obtained.

The DNA fragment between the primers is the microsatellite, which is then amplified by PCR (Polymerase Chain Reaction). The size of different amplified products (corresponding to different alleles of the microsatellite) is then determined by migration on an acrylamide gel. If the sequence is short, it will go through the gel quicly and therefore will appear at a greater distance from the "top".

This procedure is repeated for 17 different microsatellites and for every captured marmot. Genotypes obtained are then compared to each other to determine kinship links among individuals. For example, the image below shows that all the bands (corresponding to one DNA sequences from a particular size) of the offspring are present in one or both parents.



Commentaires

- Liens de parenté avec la mères : les allèles maternels se retrouvent chez l'enfant 1 (pour le vert) et l'enfant 2 pour le bleu
- Liens de parenté avec le père : l'allèle rouge se retrouve à la fois chez l'enfant 1 et l'enfant 2.

Figure 3: acrylamide gel migration

Activity 24 – HAIR PROTOCOL – Preparing samples for genetic analysis (Data sheet 13)

Complete Blood Count from captured individuals

Objective

The blood sample is used to determine the health of each individual marmot. The first step is to get a complete blood count for each individual marmot. A complete blood count, or CBC, analyzes both quantitative (number) and qualitative (types) aspects of blood components. These components include: red blood cells (erythrocytes); white blood cells (leukocytes); and platelets.
Erythrocytes are the most numerous blood cells in vertebrates. In mammals, they transport oxygen (O_2) to various body tissues. The red blood cells (RBC) pick up oxygen from the lungs, and as the blood circulates through the body, the RBC's release oxygen to the various tissues.

White blood cells, or leukocytes, are produced by bone marrow. There are several types of leukocytes: granulocytes, monocytes and lymphocytes. Each plays an important role in the immune system, which protects mammals against infectious diseases, viruses and foreign substances.

Blood cells are counted to allow us to determine the blood concentration of red cells, white cells and platelets. This count is performed by determining the hematocrit and by analyzing a blood smear. The hematocrit corresponds to the percentage of red blood cells (erythrocytes) in the total volume of blood. After staining the blood smear, the different types of white cells can be identified and counted. If a mammal has an infection or an inflammation, the number of circulating leukocytes increases dramatically.

A blood smear also helps in identifying potential blood parasites.

Principle

Counting blood cells requires just a simple blood sample. Blood samples from captured individuals are made in the presence of a buffer, to keep it "intatct". The blood sample is then diluted more or less, depending on the expected concentration of the blood cells to be counted. The expected concentration of red blood cells is greater than the expected concentration of white blood cells. The red blood cell concentration is expected to be particularly high in alpine marmots, as is the case for many mammals living at high altitudes.

The diluted blood on a microscope slide is placed in a specific device used for counting blood cells: a hemacytometer. The hemacytometer contains a background pattern of squares, which allows for a direct, precise count of cells; the background pattern prevents double counting the same cell. The hemacytometer is placed under a microscope and a photograph is taken of the arrangement of cells in the hemocytometer to facilitate counting.

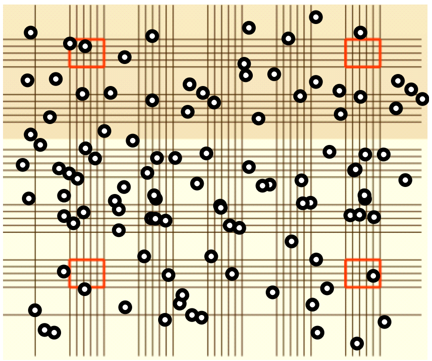


Figure 4: Cells on a hemacytometer observed under a microscope

The number of cells can then be counted on the photograph and is then compared to the counting volume, which allows researchers to obtain the concentration of each type of blood cell studied

Activity 25 – COUNTING BLOOD CELLS PROTOCOL – Establish CBC from captured marmots -step 1 (Data sheet 14)

Activity 25 – COUNTING BLOOD CELLS PROTOCOL – Establish CBC from captured marmots -step 2